THE CENTRAL NERVOUS REGULATION OF RUMINANT GASTRIC ACTIVITY

by

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ABSTRACT OF THESIS

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An extensive survey of the literature relating to the central nervous control of ruminant forestomach motility reveals that the present state of knowledge is very unsatisfactory. Although there is convincing evidence that the fundamental mechanism concerned is situated in the medulla oblongata, and that the efferent pathway from this involves the dorsal vagal nucleus, little else can be deduced.

The experiments described in this thesis, therefore, have been designed to provide more information on the location and activity of the reticulo-ruminal motor centre in sheep. Since a study of this type necessarily involves recording gastric motility, it is important that the technical and theoretical problems con-:cerned are realised. Section 1 of this thesis, therefore, is devoted to a critical survey of the methods which have been used for recording gastric motility, and of the limitations of each.

The second section of the thesis describes a series of experiments carried out in an attempt to determine the extent to which the centre is responsible for regulating motility of the forestomach - particularly of the rumen. The innervation of the rumen is described in some detail, and evidence is presented which suggests that the ventral vagal trunk does not send significant numbers of motor nerve fibres to the rumen sacs. This finding is considered in relation to the fact that after transection of the dorsal vagal trunk, motility of the rumen sacs is abolished for several days, but subsequently returns before re-innervation by the transected nerve.

The final section describes experiments carried out to demonstrate the location of the reticulo-ruminal motor centre in sheep, by determining the positions of degenerating neurons in the medulla and sensory vagal ganglia following transection of the vagal trunks. Point electrical stimulation of the dorsal vagal nucleus caused an inhibition of gastric motility - the precise area from which effects could be elicited is then described. Other responses some-:times seen on point electrical stimulation are also described. Finally, patterns of unit neuronal discharge, recorded from the dorsal vagal nucleus, are described and classified in relation to rumino-reticular motility. The findings are discussed in relation to one another, and their significance in relation to the regulation of gastric motility is considered.

Use other side if necessary.

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FOREWORD

The experiments described in this thesis were designed to provide information on the control, by the central nervous system, of rhythmic gastric motility in ruminants.

As the technical problems involved in recording gastric contraction are of a very high order, and as interpretation of the records obtained requires great caution, the first section of this work is devoted to a survey of available recording methods, and a critical analysis of the value of each.

Section 2 deals with a series of investigations carried out in the hope that they would indicate the extent to which the centre is responsible for regulating motility of the forestomach - particularly of the rumen. These involved destruction of all nerves which could be shown to supply the rumen, and determination of the effects of this on motility of this compartment.

The third section describes experiments which were carried out to demonstrate the location of the reticulo-ruminal motor centre, and some of its functional connections with other parts of the lower brain-stem. During these studies, unit neuronal activity was recorded from the dorsal vagal nucleus, and the different patterns of discharge observed have been classified, and considered in relation to traffic known to be passing down the vagus at the time the stomach is contracting.

INTRODUCTION

THE CONCEPT OF A CENTRE CONTROLLING GASTRIC MOTILITY IN RUMINANTS.

The complex morphology of the ruminant stomach, and the digestive peculiarities of ruminant animals have been appreciated for many hundreds of years. Thus Aristotle (Book II, 17, 507) writes "..... various animals, such of the horned animals as are not equally furnished with teeth in both jaws, are furnished with four such chambers" (of the stomach). "These animals, by the way, are those that are said to chew the cud.....". He goes on to describe the gross appearance of the four gastric compartments.

It is rather surprising, therefore, that no interest was shown in the central processes involved in genesis of the periodic cycles of motility which characterise the organ, until the early 1950s. Thus, it was not until 1951 that Iggo, on the basis of his observations following 'decerebration' at progressively lower levels, first advanced the hypothesis that a reticulo-ruminal centre was present in the medulla oblongata. This centre was supposed to establish, periodically, activity in excitatory vagal nerve fibres running to the stomach, and so to induce contraction. As early as (1883) Ellenberger had shown that in the sheep, unilateral cervical vagotomy did not cause any disturbance of digestive processes apart from causing transient tympany, lasting only a couple of hours; bilateral cervical vagotomy, however, abolished all gastric movements. It was suggested, on the basis of very indirect reasoning, that some gastric motility returned a few days after bilateral vagotomy - the experimental data presented support no such conclusion.

Since then, the effects of vagotomy have been re-investigated by numerous workers, including Hoflund, Mangold & Klein, Webster, Duncan, Dussardier, etc. The sum total of the knowledge gained by such experiments is that the vagus nerve provides the only significant motor innervation of the stomach. Moreover, the dorsal vagal trunk plays a considerably greater role in innervation of the rumen than does the ventral vagal trunk. These latter findings coincide with the anatomical description given by Marschall (1910), and since extended by Mangold & Klein (1927) and Habel (1956). Electrical stimulation of the vagus either of the intact nerve, or of its peripheral end following section, leads to contraction of all parts grossly innervated by the nerve in question (Marschall, 1910; Mangold & Klein, 1927).

Location of the centre.

As the vagus is the major motor nerve supplying the stomach, it might be expected that the nuclei of this nerve in ruminant animals would show certain special features. That this is in fact the case was demonstrated very clearly by Vermeulen in a series of papers between 1913 and 1915, in which close attention was paid to the morphological characteristics of the dorsal nucleus of the vagus in a series of ruminant and non-ruminant animals. From this, it emerges that in animals such as horses which possess a relatively small, undifferentiated stomach, the nucleus is poorly developed and contains only a few cells, whereas in ruminant animals the anterior part of the Nuc. dorsalis shows much more development and includes greater numbers of large neurons.

When reliable staining techniques for the Nissl substance

in neurons became available, the way was prepared for a more detailed study of the areas involved in gastric innervation by determining the distribution of nerve cells showing chromatolytic changes following section of the vagal branches to the stomach. Such studies were not in fact published until Kitchell, Stromberg and Davis delivered a short communication on the subject in 1956, describing the effects of vagotomy in calves, sheep, horses, a dog, a cat, a goat, and a pig. These findings were extended by Bell (1960) using goats and sheep, but the observations of these workers did little more than confirm the conclusion drawn by Vermeulen 50 years earlier; thus, only cells lying in that part of the dorsal nucleus of the vagus situated anterior to the obex showed any evidence of chromatolysis following section of the vagal gastric branches. More recently Szabo and Dussardier (1964) have repeated the studies following vagotomy in goats, and found that cells responsible for gastric motor innervation are scattered along the length of the dorsal nucleus of the vagus and show no localization in its anterior part.

The pathological changes present in the brain stem following the oral administration of the virus of Ausjesky's disease to sheep and calves, involve mostly the solitary tract and its nucleus, the dorsal nucleus of the vagus anterior to the obex, and other nuclei, involvement of which could be predicted on the basis of somatic sensory innervation of the upper digestive tract; no lesions were present anterior to the colliculi (Dow, 1963).

It should be noted here, however, that the validity of such methods for the determination of the central origin of nerve

fibres has been criticized (see, e.g. Mohiuddin, 1953) on the basis that they are not specific, and are rather indirect.

Electrical stimulation of the medulla oblongata.

Bell & Lawn (1955) and Andersson, Kitchell & Persson (1958) present a detailed description of those areas of the medulla oblongata, electrical stimulation of which causes contraction of the reticulum. Close examination of the results obtained by these workers leads to the conclusion that quite different structures were involved in each case. Thus Bell & Lawn were eliciting contractions of the rumino-reticulum from an area 2 mm. posterior to 7 mm. anterior to the obex from the reticular formation of the ovine medulla, and almost always located at the level through which efferent vagal fibres would be expected to pass as they run from the dorsal nucleus of the vagus to the periphery. Andersson et al, on the other hand, elicited responses from a level of 1 mm. posterior to 3 mm. anterior to the obex, and all such points lay close to the median raphé.

Dussardier (1960) also presents data showing the effects of point electrical stimulation of the medulla oblongata, and divided the area studied into 'accelerator points' (on the afferent vagal pathways), 'motor points', from which gastric contractions could be elicited, and 'inhibitory points'. Both Bell and Lawn (1955) and Dussardier (1960) claim to have elicited gastric contractions largely from the region of the dorsal nucleus of the vagus and its efferent fibres, but detailed examination of their respective data indicates that very few excitatory points were located within the nucleus itself. The relationship of the dorsal nucleus of the

vagus to gastric innervation is thus very much a matter of speculation, and it is evident that a much closer investigation of the region is necessary. In the dog, point electrical stimulation in the region of the dorsal nucleus of the vagus or electrical stimulation of the splanchnic nerves consistently caused powerful gastric contractions (Semba, Noda & Fujii, 1963). Pharmacologically at least, there is some evidence that the excitatory response to stimulation of the splanchnic nerves is due to the presence of cholinergic fibres (Kuré, Ichiko and Ishikawa, 1932; Malmejak and Donnet, 1940; Malmejak, Donnet and Monges, 1940; Malmejak, Chardon & Aubry, 1951).

Destruction of the dorsal vagal nucleus.

The problem was further investigated by Beghelli Borgatti, Mavrulis and Parmeggian's (1964), by preparing localized electrolytic lesions in the medulla oblongata of sheep. These workers found that ablation of the dorsal nucleus of the vagus on one side had no permanent effect on motility of the reticulum, whereas lesions extending from a point 3 mm. in front of the obex to 5 mm. in front, involving both nuclei, abolished rhythmic contractions of the reticulum, which had been induced by distension of the reticulum, or electrical stimulation of the abomasal nerve. Bilateral destruction of the dorsal nucleus from the level of the obex to a point 3 mm. in front of this, depressed or abolished the rhythmic contractions of the reticulum, whereas interference with the nuclei behind the level of the obex had no marked effect on gastric motility. When lesions were confined to the reticular formation of the prosencephalon, no alteration of gastric contraction cycles was seen, unless the damage extended to nerve fibres passing between the rootlets and the nucleus of the vagus nerve.

Electrical activity recorded from the dorsal nucleus of the vagus.

In 1957, Dussardier, using coarse electrodes (tip diameter 50 - 100 μ) to explore the potential field at different points along the dorsal vagal nucleus recorded slow, triphasic fluctuations in electrical potential. Such activity was only detected from a point about half way along the length of the nucleus (i.e. just anterior to the obex).

This was followed up in 1960, and 1963 by the papers by Beghelli, Borgatti and Parmeggiani, describing experiments which involved the recording of unit activity from this region of the brain stem in decerebellate, anaesthetised lambs, using platinum electrodes with a 30 μ tip. It was found that unit activity, corresponding temporally with the contractions of the reticulum, could only be detected from that region of the dorsal nucleus of the vagus extending from a level 2 mm. posterior to 3 mm. anterior to the obex. The records presented in the papers are of multi-unit preparations, usually showing 2 or 3 superimposed discharges; activity commenced 0.48 to 0.96 sec. before the reticulum began to contract and, it is claimed, with a spike frequency of 25 - 60/sec. Occasionally low frequency discharges were noted which were prolonged beyond the contraction of the reticulum and these were assumed to be derived from neurons involved in the innervation of the other forestomach compartments. It is of interest to note that unit discharges following electrical stimulation of the central stump of the transected abomasal branch of the vagus bore the same temporal relationship to contraction of the reticulum as seen in the rhythmic contractions induced by distention of the reticulum, but the discharge did not appear until 2.4 - 7.2 secs. after nerve stimulation was commenced. (It may be noted that Dussardier

and Albe-Fessard (1954) had shown that the reflex gastric contraction induced by electrical stimulation of the central end of one transected vagus nerve in the neck of an anaesthetised sheep, and occurring after a latency of 4 - 15 secs., could be abolished by electrotonic block of the other vagus, 6 secs. after stimulation.) On this basis, Beghelli et al (1963) assumed that another centre must be interposed between vagal afferent fibres and the dorsal nucleus of the vagus. This assumption, however, would appear to be premature, as the possibility of slow electrotonic changes, or of activity which was not recorded for some other reason (e.g. its restriction to nerve endings) has not been ruled out. Some units responded to mild distension of the forestomach or to stimulation of the abomasal nerve, but did not become active during contractions associated with such procedures.

Unit discharges, which showed a close temporal correlation to contraction of the reticulum, have been recorded from the diaphragm of conscious sheep after reinnervation following anastomosis of the vagus to the phrenic nerve (Dussardier, 1958; 1959; 1960; 1962). Electromyograms taken from the reinnervated diaphragm in such cases can be classified into two broad types - those showing a fairly high frequency burst lasting only about 1 sec. and beginning before the contraction of the reticulum, and a lower frequency discharge lasting 10 - 12 secs. and beginning after the first phase of the contraction of the reticulum; there was, however, too much variation to permit classification of units on the basis of any single parameter. Discharge frequencies varied between 3.5 and 20 / sec., but rarely exceeded 12 / sec. (compare these values with those obtained by Beghelli et al, 1960); in general, the rate of discharge rose rapidly to a peak, and then fell more

gradually. It was also possible to demonstrate variations in discharge frequency corresponding with the respiration rate, superimposed on activity related to that of the reticulum.

Apart from the differences in frequency of discharge, however, the unit records presented by Dussardier and Beghelli show many similarities, and may be taken as representing the pattern of nerve activity which precedes, and is directly responsible for, each contraction of the reticulum.

It is important to note here that low amplitude, localized contractile events can often be seen on the walls of the forestomach following laparotomy (See, e.g. Wester, 1926; Marschall, 1910), on X-ray (Czepa and Stigler, 1929; Phillipson, 1939) and even recorded as pressure changes from the lumen. These contractions are also seen following total vagotomy, and in the isolated stomach (Comline & Titchen, 1951); since they may appear quite independently of central nervous influence, they seem to be of intrinsic origin. Such contractions may occur irregularly between major contraction cycles, becoming more frequent as the interval since the previous major contraction cycle increases (Mangold and Klein, 1927); there is, to this extent, some relationship with the innervation - dependant motility. 'Intrinsic' motility of this type is beyond the scope of the present discussion, and its significance in normal reticulo-ruminal function has yet to be defined.

Conclusion.

From the foregoing discussion, it emerges that the dorsal nucleus of the vagus plays a prominent role in the regulation of rhythmic motility of the forestomach, although there is some doubt whether such activity arises from the entire length of the nucleus, or from a well defined region of it. Histological

studies are rather inconsistent, and the effects of electrical stimulation of the dorsal surface of the medulla remain in some doubt. Further confusion arises from the finding that the only part of the dorsal vagal nucleus from which electrical activity has been correlated closely with gastric motility is that area immediately anterior to the obex, although bilateral ablation of this region has much less effect on the contraction of the reticulum than does bilateral ablation at a more anterior level. Records of unit activity from the dorsal nucleus of the vagus have not been subjected to careful analysis, although Dussardier provides indirect data for traffic in the thoracic vagus; it would be very useful to compare such records with those obtainable directly from the vagal nucleus, especially as there does appear to be a discrepancy between such quantitative data as are available.

THE EXTENT TO WHICH THE CENTRE CONTROLS INDIVIDUAL CONTRACTION CYCLES

A topic which seems to attract attention periodically, but which has never been the subject of close investigation, is the degree to which the reticulo-ruminal centre controls events in the forestomach. When Ellenberger (1883) demonstrated the abolition of motility of the rumino-reticulum following total cervical vagotomy, it was presumed that the entire cycle was controlled by the central nervous system; authoritative publications by Marschall (1910) and Wester (1926) appear to adopt this concept without reservation although both workers recognised the ability of the musculature of the reticulum and rumen to contract independently of influences from the central nervous system.

The first suggestion that some part of the contraction cycle of the rumino-reticulum might be established at the periphery was offered by Mangold & Klein (1927) on the basis of their investigations into the mechanical events during spontaneous contractions of the reticulum, and into the response of the reticulum to direct electrical stimulation. These workers do not advance any experimental evidence for their hypothesis, which does not appear to have been followed up.

In 1948 Borgatti published an account of his own investigations into the control of forestomach motility in sheep, and asserted that although motility of the reticulum depended on influences from the central nervous system, subsequent contractions of the rumen and omasum were 'reflexly' co-ordinated by a 'centre' lying within the wall of the reticulum. This conclusion was based on the observation that, following total vagotomy, stimulation of the fundus of the reticulum, after an interval of about 3 to 5 sec. (during which time the reticulum contracted)

was followed by contraction of the cranial end of the omasum. Because of the profuse extrinsic innervation of the forestomach wall in the region of the cardia (presumably from the cardial plexus), and because the primary contraction cycles appear to originate here, Borgatti denoted this as the site of the peripheral 'centre'.

The evidence from which these assumptions are drawn is clearly inadequate and interest was not aroused again until Borgatti et al (1963, b.c) described experiments on anaesthet-:ised sheep, during which the region of the reticular groove had been surgically excised. Following this procedure, electrical stimulation of the cervical vagus still elicited contraction of the reticulum, indicating the integrity of the motor innervation to this part of the forestomach, although attempts to elicit 'reflex' contractions by afferent electrical stimulation of transected abomasal or mandibular nerves proved unsuccessful; when the region of the oesophagael groove was left intact, afferent stimulation of these nerves evoked reflex contractions. These findings cannot readily be explained in terms of damage to the sensory receptors in the vicinity of the cardia, unless integrity of these is essential for the establishment of such a contractile event in the reticulum. The effects of trauma do not appear to have been considered in relation to the findings of Dussardier (1953) and Titchen (1960) who have shown that traumatic procedures involving the forestomach may markedly depress motility of the rumino-reticulum, and that central pathways are involved in this action. It seems likely therefore that surgical interference with a region which is well supplied with receptors will cause pronounced depression of motility of the forestomach, although the response to efferent vagal stimulation would be expected to remain unaltered.

In the same year, Morrison (1960) published a preliminary report of his histological investigations into the distribution of vagal nerve endings over the ruminant stomach, and this was followed by a fuller account in the following year (Morrison & Habel, 1964). These studies, which involved histological examination of the myenteric plexus of the ovine forestomach following total thoracic vagotomy, revealed that the highest density of degenerating nerve endings occurred in a so-called "central region" which comprised the mid-dorsal rumen sac, the fundus of the reticulum, the omasal pillar and the oesophageal groove. On this basis it was suggested that a 'sub-centre' existed within the myenteric plexus, co-ordinating motility of more remote parts of the stomach; the dangers inherent in the prediction of physiological mechanisms from morphological characteristics do not seem to have been taken too seriously!

The data provided by these experiments, however, does assist evaluation of various other evidence which is advanced in support of a more complex peripheral mechanism involved in regulating the contractions of the rumino-reticulum. Brunaud et al (1959) determined intramural cholinesterase levels at various parts of the forestomach, and found that levels were statistically greater in the reticulum than elsewhere, and that concentrations fell in the sequence: dorsal sac, posterior pillar and ventral sac. Ganglion-blocking drugs tend to inhibit rumen contractions before depressing those of the reticulum (Brunaud & Navarro, 1954); the same is true of general anaesthetics (Iggo, 1954), high environmental temperature (Aliev, 1963) and various other factors which are considered later.

These findings indicate that there is some basic difference in the neuromuscular apparatus between these various parts of the

forestomach walls and suggests that more synapes are interposed between the vagus and rumen musculature than between the vagus and reticulum musculature. The observation by Duncan (1954) and Dussardier & Navarro (1953) that isolated strips of the musculature of the reticulum rarely show spontaneous motility under 'in-vitro' conditions, whereas rumen preparations do so more frequently, suggests a greater independence of the rumen musculature from central nervous influences. There is general agreement on the existance of very large numbers of ganglion cells within the walls of the forestomach, and that these are most numerous and most densely packed in the region of the oesophageal groove (Kolassov, 1933; Grau & Walter, 1957; Rosatti & Pelagalli, 1960), where Kimata (1960; 1963) has shown the highest levels of mono-amine-oxidase activity, but the significance of this remains obscure.

Perhaps the strongest evidence which can be advanced to support the hypothesis that there is a subsidiary centre located in the stomach wall, is derived from experiments involving section of the vagus nerves. The innervation of the stomach by the vagus has been described by Marschall (1910), Mangold & Klein (1927), Mangold & Klein (1927), and more recently by Habel (1956). It appears, from these descriptions, that the rumen is supplied exclusively by the dorsal vagal trunk, whereas all other compartments of the stomach are innervated by dorsal and ventral vagal trunks. Habel describes a few fibres crossing between dorsal and ventral vagi, posterior to the diaphragm, but these are not mentioned by the other two workers; moreover, Habel failed to demonstrate the presence of degenerating fibres in the ventral abdominal vagal trunk following section of the dorsal thoracic trunk, nor did he detect degenerating fibres in the dorsal abdominal trunk when the ventral thoracic vagus was cut.

If there is an interchange of nerve fibres between the two vagal trunks in the abdomen this does not appear to involve myelinated axons.

In view of the fact that very little of the rumen innervation is derived from the ventral vagal trunk, perhaps it is not surprising that section of this nerve causes little alteration in the pattern of rumen motility - see for example Duncan (1953), Bell (1960), Dussardier (1961) and Kuz'min (1963). These workers also report, however, that section of the dorsal vagal trunk does not lead to the complete disappearance of rumen motility - Dussardier alone claims that the health of his experimental animals (young sheep) was significantly impaired, but he was unable to determine whether the rumen still showed contractions. Section of both vagal trunks leads to complete atomy of the forestomach (Hoflund, 1940; Duncan, 1953; Kuz'min, 1963).

The available evidence, therefore, appears to suggest that although the ventral vagal trunk does not innervate the dorsal or ventral sacs of the rumen, it is capable of supporting rhythmic, contractile events of this forestomach. This suggests that either:-

1. The reticulum could, in some way, lead to a contractile event of the rumen, immediately following its own contraction, by some purely peripheral mechanism.

or

2. There is some overlap of innervation - e.g. postganglionic nerve fibres may run outside the areas from which they arise, so that return of motility could be due to degeneration hypersensitivity.

Neither hypothesis has independent justification.

Duncan (1954) showed that after section of both thoracic vagi in three sheep, the intravenous injection of 2 mgm. acetylcholine

chloride did not cause the rumino-reticulum to contract; spontaneous motility was abolished. In the same animals, intravenous injection of 0.3 to 1.5 mg. L-adrenaline, or 1 mg. L-arterenal caused a single, slow contraction of all parts of the stomach; because the same observation was made when gastric contractions had been abolished by anaesthesia with sodium pentothal or by atropine, it is unlikely that this is due to degeneration hypersensitivity. Pharmacological evidence does not support the hypothesis that following vagal section, there is an increased sensitivity of the forestomach to adrenaline or acetylcholine. In contrast to this, Iggo (1954) has shown a greatly increased sensitivity of the reticulum to cervical vagal stimulation following section of the contralateral cervical vagus. This finding indicates that degeneration hypersensitivity does occur in this compartment at least.

THE PHYSIOLOGICAL PROPERTIES OF THE RETICULO-RUMINAL CENTRE.

Although the precise location of the reticulo-ruminal centre seems a little uncertain at present, a number of its properties have been investigated.

Excitatory effects on motility from the digestive tract.

It has been mentioned that contractions of the reticulo-rumen can be modified by afferent activity from a large number of sources. Thus, the induction of rumination by tactile stimulation of the anterior of the rumen is well established (Schalk & Amadon, 1928; Bost, 1958; Ash & Kay, 1959; Salmin, 1960). Distension, with gas, of the dorsal sac of the rumen of conscious sheep, leads to the appearance or potentiation of 'secondary' rumen contractions (Wester, 1926; Weiss, 1953); such responses can also be elicited in decerebrate sheep (Dougherty et al, 1958; Titchen & Reid, 1965; Reid & Titchen, 1965), and even following section of the medulla oblongata at the level of C.1. (Dougherty et al, 1958). These secondary contractions of the rumen differ from those which occur in the primary cycle, in so far as the rise in pressure within the rumen first becomes apparent in the

caudoventral blind sac of the rumen and thereafter can be recorded successively from the caudodorsal blind sac and at progressively more anterior levels in the dorsal sac of the rumen (Reid, 1963).

Reflex aspects of closure of the oesophageal groove are summarised in a paper by Comline & Titchen (1961), although it is perhaps rather dangerous to regard the physiological characteristics of the striated musculature of this region, with its end-plate innervation, as typical of gastric musculature in general. Thus, Titchen (1954), pointed out that following splanchnotomy, distension of the abomasum leads to an increase in the frequency of contraction of the reticulum in decerebrate, adult sheep and goats, whereas in the decerebrate calf, distension leads to inhibition of the reflex responsible for contraction of the lips of the oesophageal groove.

In view of the variation between rumen contractions, when compared with those of the reticulum, it is unfortunate that so little attention has been paid to motility patterns of the forestomach as a whole. Most workers who have investigated the effects of afferent stimulation of various types on ruminant gastric motility recorded only pressures from the lumen of the reticulum. It is dangerous to relate such information to gastric motility in general, particularly when observations have been made on anaesthetised or decerebrate animals, since preparations such as these rarely show spontaneous rhythmic motility of the rumino-reticulum, and this only appears in a very simple form following suitable stimulation (e.g. by distension of the reticulum or lower oesophagus, stretch of the rumino-reticular fold, acidification of the abomasal contents, or weak electrical stimulation of the central end of a sectioned vagal trunk) (Iggo, 1951; Titchen, 1953, 1958, 1960; Dussardier, 1955; Borgatti et al, 1963 a, b).

Factors which depress motility.

The ease with which motility established under such conditions can be suppressed, makes complex experimental techniques rather impracticable. Moderate distension of, or trauma to, the abomasum causes a marked depression of motility of the reticulum, an effect which is completely abolished by total splanchnotomy, unless the stimulus involves the pyloric region, when the effects of splanchnic section are less pronounced (Wester, 1926; Dussardier, 1955; Titchen, 1958). The inhibitory effect on rumen motility is even more marked (Titchen, 1960). Makaleev (1961) reports that the inhibitory effects of abomasal distension on gastric contractions in conscious sheep are not reduced when Novocaine is topically applied to the splanchnic nerves; this worker does not record the position of the distending balloon within the abomasum, but it may have been situated near the region of the pylorus. On release of the distension, motility was often enhanced, and rumination was sometimes seen. Splanchnotomy in the decerebrate, anaesthetised or conscious animal is either followed by an increase in strength of reticulum contractions, or has no demonstrable effect on gastric motility (Duncan, 1953; Titchen, 1958, 1960).

In general it appears that any procedure which depresses motility of the rumino-reticulum exerts a greater effect on the rumen than on the reticulum - this observation extends to the action of ganglion-blocking agents (Brunard & Navarro, 1954), general anaesthetics (Iggo, 1954) and even to exposure of conscious animals to high environmental temperatures (Aliev, 1963). Local trauma to the rumen (Dussardier, 1953; Titchen 1960), intestinal tract (Trifanov, 1960); Nekrasova, 1961) or abdominal wall (Titchen, 1960; Dedashev,

1959) has a less pronounced inhibitory action on contractions of the rumino-reticulum. Titchen (1960) showed that the inhibition of motility following traumatic manipulations of the forestomach was abolished by high cordotomy; this is further evidence that afferent fibres of the sympathetic nervous system may inhibit the reticulo-ruminal centre.

Another inhibitory input to the reticulo-ruminal centre is provided by the mandibular branch of the trigeminal nerve; in lambs anaesthetised with chloralose, stimulation of the central stump of the cut nerve causes a reduction in amplitude of rhythmic contractions which are being maintained by stimulation of the abomasal branch of the vagus (Borgatti et al, 1963). Under such conditions, especially when the stimulus strength delivered to the mandibular nerve is high, the inhibition is preceded by an additional contraction of the reticulum (see also Borgatti & Matscher, 1956, 1958); this had originally led to the idea that the fundamental response was one of excitation, but it is now quite clear that both inhibitory and excitatory components are involved. A similar relationship has been shown on stimulation of the central stump of the cut sciatic nerve in curarised or anaestherised sheep following bilateral splanchnotomy (Dussardier, 1955); stimulation at 3 V and 50 c.p.s. causes the appearance of, or an increase in the frequency of reticular contractions, whereas further increase in the voltage tends to lead to a depression of the frequency of contractions when these are present. This may suggest that the inhibitory component is carried by nerve fibres of smaller diameter than the excitatory effect.

<u>The manner in which the peripheral input could modify activity of the centre</u>.

Activity from these various sources need not necessarily

act directly on the centre. In fact, the variations of response in each case, and the long latency involved before the effects become apparent suggests rather that their action is non-specific, in much the same way that Delisle Burns & Salmoiraghi interpret the afferent activation of the respiratory centre. If this were to be the case, it would account for the complex inter-relations between somatic and autonomic nervous systems, and it would also account for the findings by Beghelli et al (1963) and Dussardier & Albe Fessard (1954) that there is a long central delay between afferent stimulation and the appearance of a motor response. An electrophysiological basis for such a belief has recently been provided by Too & Dussardier (1963) who recorded unit activity from the reticular formation of the medulla oblongata in anaesthetised goats and sheep and were able to show that a given cell could be influenced by activity in both the vagus nerve and somatic afferent nerves (median and tibial nerves) of either side.

Influence on the reticulo-ruminal centre of other parts of the central nervous system.

Although the influence of peripheral factors on gastric motility has been studied by numerous workers, the control exerted from other parts of the encephalon has received very little attention. It has been mentioned that under conditions of mid-collicular decerebration, or even light anaesthesia, rhythmic contractions of the rumino-reticulum rarely occur spontaneously, although they may be caused to appear by increasing the excitatory sensory input to the centre. These findings appear to indicate that other parts of the central nervous system are capable of influencing the reticulo-ruminal centre, although their precise role cannot be absolutely defined at present. It is apparent

from the findings of Dussardier & Albe Fessard (1954) that all essential components for the establishment and maintenance of rhythmic contraction cycles of the reticulum are left intact following section of the brain stem 5 mm. behind the posterior border of the pons.

THE CENTRAL CONTROL OF RUMINATION

Perhaps the aspect which has attracted greatest interest is the change in motility pattern which is associated with rumination. Classically, this change is described as an increase in rate of the reticulo-ruminal cycles, and the development of a third contractile event in the reticulum preceding the two commonly recorded from this viscus (Czepa & Stigler, 1929; Wester, 1926; Schalk & Amadon, 1928; Slanina, 1958). More recent studies (Bost & Ruckebusch, 1962) have suggested that the sequence of gastric contractile events during rumination may be considerably more complex than had been initially imagined; thus, there appears to be a considerable degree of species variation (Fujioka & Iwata, 1958; Dzuik et al, 1963), and even in a given animal, records of intra-reticular pressures may show a high degree of variation during successive rejection cycles. Further confusion is added by reports from some workers (e.g. Bost, 1958; Bost & Ruckebusch, 1962) that there is no 'additional' contraction of the reticulum, only a separation and 'differentiation' of events occurring in the non-ruminant state. A more detailed discussion of the mechanical events associated with rejection would involve considerable further elaboration, and is largely irrelevant to the present discussion. The point of importance is that these authors are basically agreed that spatial and temporal relation-:ships of contractions recorded from the reticulum are modified by the act of rumination.

This implies that the rhythmic activity of the reticulo-ruminal centre can be modified during the act of rumination, or alternatively that there is a second 'rumination' centre which can become active at the appropriate time, and which is able to over-ride the other centre. Very little experimental work has been done to assist in the choice between these two alternatives. Experiments by Andersson et al (1958) have already been mentioned; it was found that in conscious goats, point electrical stimulation of the dorsal aspect of the medulla, about 1 mm. posterior to the obex, could induce rumination, whereas stimulation of the reticular formation medially to the root filaments of the hypoglassal nucleus augmented gastric motility, and on prolonged stimulation rumination sometimes appeared. At first sight this might appear to suggest that two separate centres are involved, but when the complexity of afferent inputs to the central regulating system is taken into account, it is not surprising that such results should be forthcoming.

The fact that manipulation of the udder can induce rumination has long been recognised (Czepa & Stigler, 1929; Stigler, 1930) and Grachev (1952) has shown the response to be abolished following section or local blocking of the nerve supply to the teats. Val'dman (1959) showed that section of the dorsal spinal roots in lactating goats, and division or blocking (with chronically implanted thermodes) of the dorsal columns of the spinal cord, abolished the effect. There seems no justification, in this case at least, for assuming the involvement of afferent pathways to the medulla other than those concerned with the mediation of somatic sensation.

'Rumination' and 'Non-rumination' contraction cycles of the forestomach show very similar time relationships and similar

pressure changes at different points within the cavity (Bost & Ruckebusch, 1962) so that the mechanical events caused by two such centres appear to be very similar. Indeed, if separate centres are to be postulated, one would be quite justified in assuming that a third centre regulated motility during feeding, when the frequency of reticulum contractions and the relative number of secondary to primary contraction cycles rises consid-:erably (Schalk & Amadon, 1928; Weiss, 1953; Nesic, 1960; Freer et al, 1962) and motility in the ventral sac of the rumen changes markedly (Phillipson & Reid, 1963). One is then presented with the difficulty of explaining the transitional forms between all three types of motility.

The concept of a separate centre.

Electrical stimulation of the hypothalamus in conscious goats induces 'drowsiness' and sometimes also rumination (Andersson, 1951; Larsson, 1954). Bell (1959) has concluded on the basis of this evidence, and because rumination is often associated with milking, that the hypothalamus is involved in regulating the activity of the reticulo-ruminal centre. This is a difficult standpoint to justify, because it has been very well established that point electrical stimulation of various parts of the brain stem, including the hypothalamus, leads to a marked behavioural change and may even be used in conditioning experiments in laboratory animals (for a review of this subject see Olds, 1961). It has been recognised for a great number of years that rumination is almost invariably associated with quiet, comfortable surroundings, and with a distinct behavioural pattern (see, for example, Wester, 1926; Czepa & Stigler, 1929; Stigler, 1930). The hypothesis that the appearance of rumination on stimulation of the hypothalamus was secondary to such changes in demeanour is supported by the fact that on electrical stimulation behavioural

changes frequently occur before rumination appears (Larsson, 1954).

It is sometimes suggested that rumination is established by a mechanism similar to that involved in the phenomenon of sleep (Bell, 1959). This supposition is based on the fact that ruminants are said not to require sleep (Balch, 1955), that the daily period spent ruminating resembles that spent sleeping by non-ruminant animals, and that electroencephalograms from sleeping and ruminating animals show many similarities (Bell, 1958). Closer investigation of the e.e.g. shows, however, that there are a number of differences between that recorded during sleep and that during rumination; for example, the spindling pattern characteristic of deep sleep does not appear during rumination, and there is behavioural and electromyographic evidence that ruminants do periodically show activity resembling that seen during sleep (Salmin, 1960; Ruckebusch & Bost, 1962). In view of the complex nature of those mechanisms involved in the establishment and maintenance of sleep, it does not seem likely that the same mechanism is involved in each case; both Salmin (1960) and Ruckebusch & Bost (1962) noted that 'sleep' was interrupted before rumination appeared.

Another hypothesis which has been advanced to explain the existence of a central mechanism, especially adapted to establish the complex set of events involved in rumination is that the vomiting centre has been modified to accommodate this act. This reasoning is based on the false premise that ruminants do not vomit (see, for example Fox & Fincher, 1956; Andersson et al, 1958 b; Blood & Henderson, 1960). Arduini & Dagnino (1953) suggested that the vomiting and rumination centres were identical, since administration of large doses of apomorphine to sheep, increased rumen motility, and was sometimes followed by chewing movements which these workers described as 'rumination'. Andersson et al (1958 a) failed to confirm these

findings in the goat, although after administration of apomorphine rumination could still be elicited by tactile stimulation of the udder. As early as 1949, Stigler had critically compared the mechanics involved in rumination and vomition, and demonstrated that quite different mechanical events are involved in each case. There seems to be no justification for the assumption that the central mechanisms for rumination and vomiting are identical.

Central processes involved in the control of rumination.

Although it is not possible to identify the 'rumination centre' with any centre recognised in non-ruminant animals, it is possible to indicate several other centres which can show functional liaison to it. The initial events in the act of rumination appear to occur in a pre-ordained sequence, particularly those occur-:rences associated with rejection thus, if the rumino-reticulum is completely emptied, tactile stimulation of the mucosa in the region of the reticulo-omasal orifice or reticular groove is followed by dilation of the cardiac orifice, movement of the diaphragm associated with the drop and subsequent rise in intra-thoracic pressure, oesophageal antiperistalsis and even two or three chewing movements (Wester, 1927; Bost & Ruckebusch, 1960, 1962). After transecting the oesophagus, so that material rejected from the rumino-reticulum fails to reach the mouth, masticatory movements resembling those seen during normal rumination still apear (C. Foa, cited by R. Stigler, 1930). If no material enters the pharynx, however, mastication is terminated after only a few seconds; thus, it appears that, unless additional stimuli become available, during the act of rumination, the sequences involved are terminated before completion. Shortly after deglutition (presumably of saliva which has collected in the mouth and pharynx) the act may be repeated; this cycle of events may be repeated several times. These findings accord with what would be expected to occur if sensory information

from the upper part of the digestive tract exerted a 'positive feed-back' on the centres controlling rumination, to modify their output. In this connection, it is perhaps significant that Bergman & Dukes (1926); Bost (1958) and Bost & Ruckebusch (1962) observed that the diaphragm contracted several times during rejection if material failed to enter the abdominal part of the oesophagus, whereas under normal conditions, when some of the contents of the rumino-reticulum were aspirated, only a single contraction was seen; in this instance, then, the entry of food into the lower oesophagus could be causing a 'negative feed-back' mechanism to suppress activity of the diaphragm. Moreover, it is evident that the apparatus responsible for co-ordination of the mechanical events involved in rumination must be able to correlate motility of the diaphragm, larynx, oesophagus (and the muscles involved in mastication) with the rhythmic contractions of the reticulum, to which they bear a constant temporal relationship. It is not possible, at the present state of knowledge, to indicate by what manner the activity in these different organs is modified, but in view of the great diversity of function involved, it seems probable that the activity of the respective centres is altered in some way.

Ruckebusch (1963 b) was able to demonstrate that the rumination induced by tactile stimulation of the interior of the rumino-reticulum could be 'conditioned' to a series of flashes and clicks delivered one minute earlier; it was also possible to elicit a conditioned reflex inhibition of rumination to the verbal command 'Stop', but training in this latter case took considerably longer than reflex excitation. This susceptibility of the 'rumination centre' to conditioning is shown also by the reticulo-ruminal centre, since the increased frequency of rumino-reticular contraction cycles which is present during feeding (see p. 12)

can be conditioned to the sound of a bell (Ruckebusch, 1963 a); the depressed motility of the ovine rumino-reticulum which follows distension of a balloon in the rectum, or an electrical shock, has been conditioned to the sound of an electric bell (Dedashev, 1959). Results such as these are sometimes taken to indicate that the cerebral cortex exerts a direct regulating influence on the centre, or centres involved. This reasoning, however, depends on the assumption that 'learning' processes are confined to the cerebral cortex; this basic premise has not been shown to be true.

Clark (1953) performed frontal lobotomy on two sheep and prepared localised lesions of this region in the encephalon of other sheep, and was able to demonstrate that following such procedures the time spent ruminating was much longer than it had been previously. Similar experiments involving ablation of the lobus frontalis in five goats have been carried out by Bell & Lawn (1956), but under these conditions it was found that the normal rhythm of rumen movements and the act of rumination, were replaced by irregular periods of remastication. Post-mortem controls in these experiments do not seem to have been very precise, and the regions destroyed seem to have varied in different experimental animals; thus, the difficulties which one normally encounters in interpreting experiments involving the ablation of relatively large areas of the telencephalon, are considerably magnified.

From a behavioural view-point, however, it does seem possible that the act of rumination can be suspended "at will", as when the animal takes an apparently spontaneous interest in its surroundings (Wester, 1926); the drawing of inferences from observations of this type is a matter requiring great caution,

and until more satisfactory evidence becomes available, it is perhaps more prudent to treat, as unproved, the concept of a 'regulatory' influence exerted by the telenecphalon over the rumination centre.

THE CENTRAL CONTROL OF ERUCTATION

A consideration of these factors thus leads to the conclusion that during rumination the activities of many centres situated in the medulla are modified; to some extent this occurs in a pre-ordained sequence. Certain stages can be altered in a manner which suggests 'positive' and 'negative' 'feed-back' on the different centres concerned. Influences from the telencephalon may be able to interrupt re-mastication, and to initiate or prevent the act of regurgitation (Wester, 1926), but further information is required before the significance of such observations becomes apparent.

Considerably less is known about the central control of eructation. From the mechanical point of view, eructation involves co-ordinated movements of the rumino-reticulum, diaphragm, oesophagus, larynx, pharynx, and other parts of the respiratory apparatus. A detailed description of the mechanical events involved in eructation would be out of place here, but most of the factors involved are considered by a series of papers by Dougherty and his colleagues: Dougherty et al, 1958; Dougherty & Habel, 1955; Dougherty & Meredith, 1955; Dougherty et al, 1962 a; Dougherty et al, 1962 b. Virtually nothing is known about the co-ordination of these different organs, although all necessary functional connections appear to lie between the level of the colliculi and the level of C 1, since isolation of the remainder of the central nervous system does not abolish eructation which is induced by inflation of the rumen with gas (Dougherty et al, 1958). The reflex aspects of eructation have been demonstrated by Dougherty et al (1958), and involved tension receptors in the region of the cardia, activation of which tended to increase the frequency of

eructation; additional receptors detect the presence of liquid as distinct from gas, in the region of the cardia, and the afferent activity from these depresses the frequency of eructation.

CHEMICAL AGENTS WHICH CAN MODIFY ACTIVITY OF THE RETICULO-RUMINAL MOTOR CENTRE.

So far, in considering the properties of the reticulo-ruminal centre, attention has only been directed toward those features which can be expressed in terms of neural input and output. Certain chemical agents are capable of altering the amplitude of gastric contractions by a direct action on the rumino-reticulum. Thus adrenaline, injected intravenously, lowers the amplitude of spontaneous contractions of the forestomach, but a similar response is seen if the contractions are induced by efferent electrical stimulation of the vagus nerve, (Brunaud & Navarro, 1953 a) and hence the inhibitory action must be exerted peripheral to the point of stimulation, i.e. it is not a direct effect on the centre. Using techniques such as this, Brunaud & Navarro (1955) demonstrated that intravenous infusion of sodium, potassium, ammonium or calcium chloride solutions in low doses enhanced the contractile response of the rumino-reticulum to vagal stimulation, whereas in higher doses the amplitude of contraction was reduced. It is evident that such agents could modify gastric motility without necessarily affecting the reticulo-ruminal centre directly. Alterations in the frequency of spontaneous contractions, however, must indicate that the factor concerned is acting directly or indirectly on the reticulo-ruminal centre, since it is the periodic discharge of the latter which determines the rhythm of motility adopted by the forestomach.

In adult sheep the intravenous infusion of a glucose solution in doses of 0.2 to 1 gm/Kilo causes a reduction in the frequency and amplitude of contractions of the forestomach which tends to become more pronounced as the blood sugar levels increase (Le Bars et al, 1953). Conversely, injection of insulin is

followed by an increase in the frequency and amplitude of rumino-reticular contraction cycles which varies with the fall in blood glucose concentration (Le Bars et al, 1953; Bowen, 1962). Similar experiments have demonstrated that increases in blood urea, or ammonium acetate levels are also associated with reduction of the frequency of contraction cycles (Le Bars et al, 1957). There is, at present, no evidence to indicate whether the changes in amplitude observed in such cases result from alterations in the output from the centre, or whether more peripheral factors are involved, as mentioned earlier. It is, moreover, impossible to decide on the basis of published data, whether the frequency of discharge of the centre is modified directly or indirectly; little will be gained, therefore, from a more detailed investigation of these aspects of the problem at this stage.

It is, perhaps, of some significance that intravenous injections of anticholinesterases such as Neostigmine, Eserine, or 309C (Brunaud & Navarro, 1953 b; 1953 c) lead to an increase in the frequency of contractions of the rumino-reticulum, and in higher dose contractions of the rumen at least become very frequent and tend to fuse together. Similar effects are seen when carbachol is administered systemically (Duncan, 1954), and high systemic doses of acetylcholine do not affect the gastric response to stimulation of the vagi, but they do inhibit contractions which were being evoked by electrical stimulation of the central stump of a cut vagus nerve (Dussardier, 1954). Findings such as these, taken in conjunction with comparative evidence provided by the histochemical studies of Lewis & Shute (1959) for the rat and the pharmacological studies by Benetato et al (1961) on the chemical substances released from the rhombencephalon during afferent stimulation of the vagus nerves of cerveau isole dogs,

suggest that acetylcholine could be involved as a chemical transmitter substance in the dorsal nucleus of the vagus. If this should prove to be the case, many of the results from experiments involving systemic administration of choline esters and similar substances to ruminants would be easily explicable.

Felinski et al (1959) have shown that contractions of the rumen (recorded as pressure changes, using a balloon and Marey tambour) in adult sheep, show fluctations in amplitude and frequency at different times of the day; the frequency of contraction was highest between 11 a.m. and 1.0 p.m., and lowest between 5.0 and 7.0 a.m. Fresh analysis of the data presented by Fujioka & Iwata (1958) shows that the number of rumination cycles made by cattle and, somewhat less clearly, by goats, showed a peak at about midnight, and was at its lowest frequency between 9.0 a.m. and 5.0 p.m. Further data on the occurrence of a diurnal rhythm in the motility pattern are clearly required, but if, as seems likely, contractions are more frequent at certain times of the day than others, it may be necessary to pay more attention to fluctuations in blood hormone or metabolite levels than has previously been the practice.

SECTION 1

RECORDING METHODS

In this section, the various methods available for recording gastric contractions in ruminants will be critically reviewed. It is felt necessary to discuss the problems involved at some length, as the interpretation of the records obtained by each method requires great caution, and a particular arrangement may prove suitable for one purpose, but not for another. The methods adopted in this thesis are detailed at the beginning of the relevent sections.

The problems of recording contractions of a large, hollow viscus such as the ruminant stomach, are numerous and complex. Contraction waves, for example, might arise from any part of the viscus, and they might travel by different routes, as for example, do the so-called "primary" and "secondary" rumen contraction cycles, the former arising directly after the appearance of a reticulum contraction, and spreading backward to involve the posterior sacs, and finally the anterior ventral sac. Secondary cycles arise in the region of the posterior ventral sac of the rumen and from here propagate forward very rapidly, but decrementally to involve the dorsal rumen sac, and later also the rest of the ventral rumen sac (Slanina, 1958; Seren, 1959; Reid, 1960). Since these latter are not temporally related to a preceding reticular contraction, simultaneous recording of the movements of both reticulum and rumen does allow differentiation on a basis of inference, but it is known that all contractile events do not follow the same course, and ideally, contractions should be recorded from a number of points simultaneously, as was done by Chiesa, Vacirea & Colombo (1962). This, however,

involves the use of a large number of recording channels, and is accordingly rather cumbersome and inflexible for general use.

The methods available for the detection of gastric contractions are divisible into 5 groups as follows:-

1. Determination of movements of the wall
   a) the muscle may be left intact
   or
   b) a strip of muscle may be dissected partly free.
2. Measurement of changes in tension of the wall.
3. Detection of electrical activity associated with the contractile events.
4. Ausculation.
5. Recording of pressure changes within the lumen of the viscus.

Of these, only 2 is a direct indication of muscular activity, while 1 reflects the movements as they are translated by the large, complexly arranged layers of muscle fibres; 4 and 5 are secondary to the contraction.

1. Movements of the wall.

The stomach may be left intact, as in the studies by Slanina, (1960), and others, who simply determine movements of the body wall. Precise control is not possible using these methods, and traces obtained represent an integrated event in the underlying viscus. If surgical interference is necessary to bring the appropriate part of the stomach to a position where movements of its wall can be detected through the skin, as in the 'herniation' procedures adopted by Reid, (1963); Comline & Titchen, (1951) etc., it is necessary to allow for the effects of this, both on local mechanical restraining factors, and on passive changes imposed by the contraction of other parts of the stomach wall.

X-ray techniques overcome many of the problems involved in making the walls accessible to observation; the outline of the organ can be displayed after administration of a suitable contrast agent, or the implantation of lead shot in the muscle of the stomach. The method suffers, however, from the poor contrast available in such relatively large animals, and the difficulties in interpretation of events when an organ is partly concealed by another (e.g. the omasum).

The technique of direct inspection of the stomach, after laparotomy, was developed by Mangold and Klein, (1927), using lightly anaesthetised or (as they used most frequently) fully conscious animals; the techniques should provide a fairly accurate indication of events in those parts of the stomach which can be easily observed. This method is probably the most satisfactory from a point of view of determining the contractile sequences in the cycle, but suffers, like radiographic or endoscopic methods, from the difficulty of quantitation. On a chronic basis, perspex windows could be set into the abdominal wall, but this method does not seem to have been developed in ruminants.

Schalk and Amadon (1928) and Wester, (1926) preferred to illuminate the interior of the rumen after fistulation, and to examine movements in this way, but unfortunately it is usually necessary to empty the forestomach, a process which might be expected to modify events appreciably; the view of different parts is rather restricted. These objections may be partly overcome by the use of a periscope system such as that described by Albright, Davis & Blosser, (1963).

Palpation of the interior of the forestomach, via a large chronic fistula - also adopted by Wester (1926) - allows exploration of the whole of the rumino-reticulum, without the need for emptying the stomach; quantitation is an even greater

problem, and the method probably has very little application.

Reid & Cornwall (1959) attempted to introduce rather more precision to the recording of gross movements of the wall by recording changes in vertical height of different points on the forestomach wall by means of a manometer and electro-mechanical transducer. Their method, however, is not suitable for recording in a horizontal plane, and therefore interpretation is hazardous, especially as movements of different parts of the stomach may be passively imposed onto the point under investigation.

Comline & Titchen (1951 b) approached the problem by dissecting a slip of muscle free from the stomach wall and recording this. It seems rather dangerous to suppose that the movements of this will accurately follow those of the intact wall, or that movements of the surrounding musculature can be entirely eliminated, although their approach could allow a differentiation of effects in different layers of the muscularis.

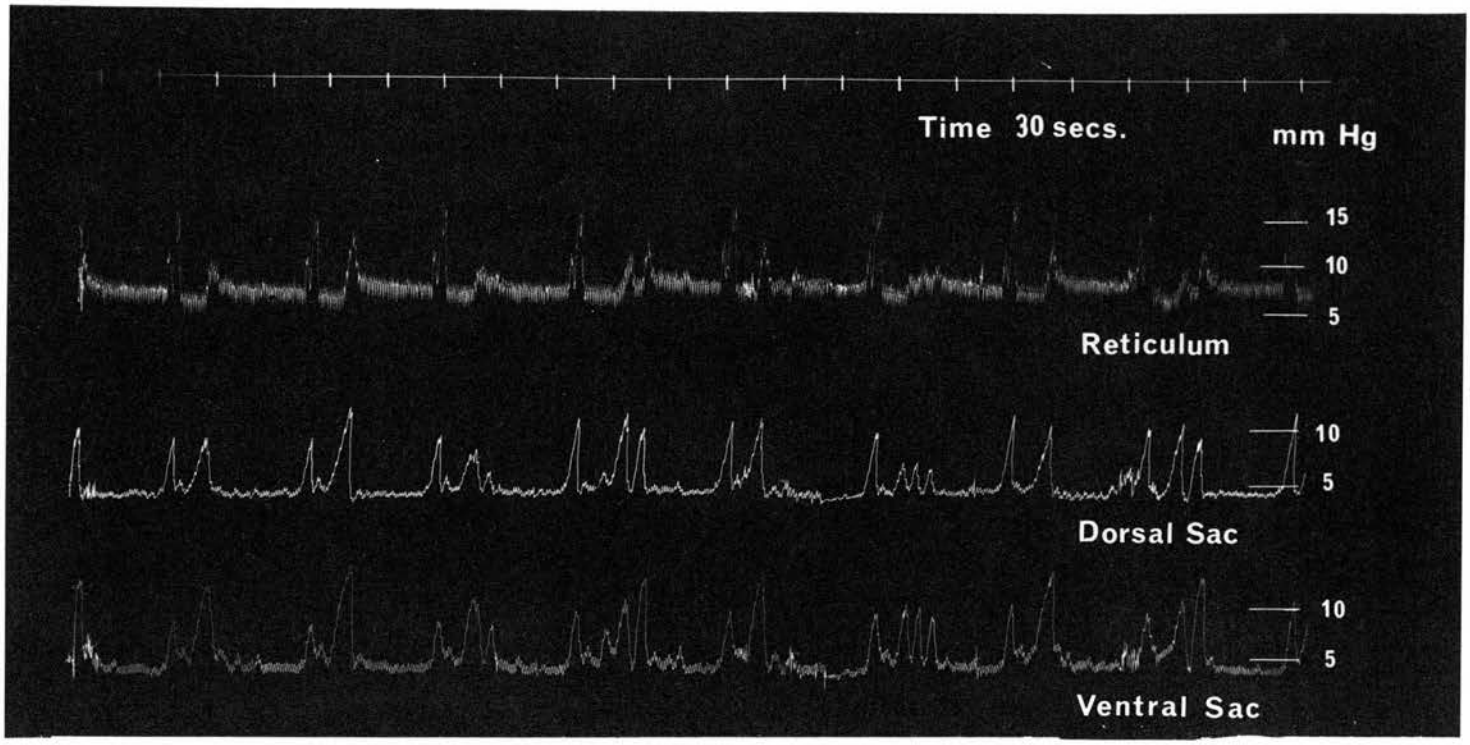
2. Changes in tension of the wall.

Although this is the only direct recording method, it determines events only in the immediate vicinity of the sensing device, and hence, as mentioned earlier, it is theoretically necessary to arrange for a number of sensing heads to be arranged at different points on the wall. The larger the sensing head, the greater will be the area examined; the trace will then represent an integration of events - e.g. the passage of a contractile wave. To a large extent this is unavoidable, since the muscle coats are arranged in different directions, and hence may become active at different moments, so that even the precise definition theoretically available is severely limited.

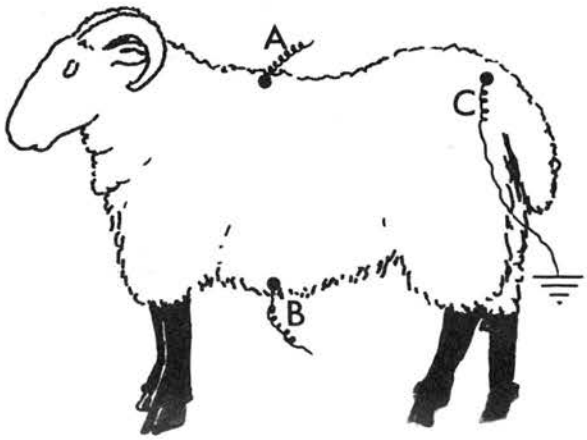
3. Electrical activity

One method of recording gastric activity which has received very little attention is the determination of fluctuations on electrical potential associated with contraction of the rumino-reticulum. This technique was first proposed by Van der Heyde (1927) and was subsequently described in more detail by Habisashi (1964 a,b; 1965). As very little surgical interference is involved, and as the comments made by earlier workers seemed fairly encouraging, it was decided to determine the value of such recordings as an indication of gastric contractile events. An adult Scottish Blackface sheep with a fistula of its dorsal rumen sac was restrained in a metabolism cage. Records of pressure changes at various points within the rumino-reticulum were made by inserting balloons, which were connected by stiff polythene tubing to Marey tambours, events being recorded on the kymograph (a fuller account of the experimental techniques is given in section 2). A portable Devices e.c.g. machine, converted for D.C. operation, was used to demonstrate the voltage fluctuations across the abdomen, at different positions. The locations of the electrodes were varied until the electrical signal received was maximal and the e.c.g. deflection was minimal - the definitive positions are indicated in Fig. 1. It is apparent from the records presented in Fig. 1. that the electrical signal is subject to considerable variation from cycle to contraction cycle, and that these variations do not correspond clearly to variations in pressure events, determined simultaneously. In view of these findings, and because the recordings obtainable do not appear to present as much intelligible information as can be obtained from the kymograph, it is felt that more investigation of the technique would be desirable before its definitive adoption.

Fig. 1. Evaluation of the electro-rumenogram as an index of rumino-reticular motility. Kymograph tracing shows pressure recording from within reticulum and dorsal and ventral sacs of the rumen. The electro-rumenogram was taken from the positions shown on the sheep, simultaneously with the upper tracing. Each contraction of the reticulum is associated with a 7-8 mV biphasic fluctuation of the electro-rumenogram tracing, but other components of the cycle, and secondary rumen cycles are not represented.



Electro-rumenogram



4. Auscultation

Although widely used clinically, and also adopted by early workers in ruminant physiology, notably Ellebberger (1883) this procedure is capable of indicating little more than the presence or absence of movement in the contents of different stomach compartments, and hence, the information provided is inadequate for physiological investigations.

5. Changes in pressure within the lumen.

These provide a very indirect approach to determination of contractile events in the musculature of the stomach, but have been very widely adopted as an absolute indication of gastric motility. It is a relatively simple matter to separate pressure changes arising from contraction of the walls from those imposed by general variations in intra-abdominal pressure; this may be done by differential manometry, or by recording events from within the rectum (see e.g. Stigler, 1930), or abdominal cavity. However, as Reid & Cornwall (1959) point out, although pressure recording can indicate movements of the walls of a compartment, in the vicinity of the sensing device, it fails to indicate movements of the pillars etc. Pressure events do not appear to be transmitted over a great distance in the rumen - thus Bost (1958) records that a pressure drop in the region of the cardia at the time of rejection cannot be detected in the fundus of the reticulum, and Bost & Ruckebusch (1962) comment on the great variability of their pressure traces when recorded from different points in the reticulum.

To provide further evidence on this point, an adult Blackface ewe was provided with two chronic fistulae of the dorsal sac of the rumen, one situated in the anterior dorsal sac, the other 14 cm. posterior to this. Balloons were inserted into the reticulum, anterior and posterior parts of the dorsal sac of the rumen, and

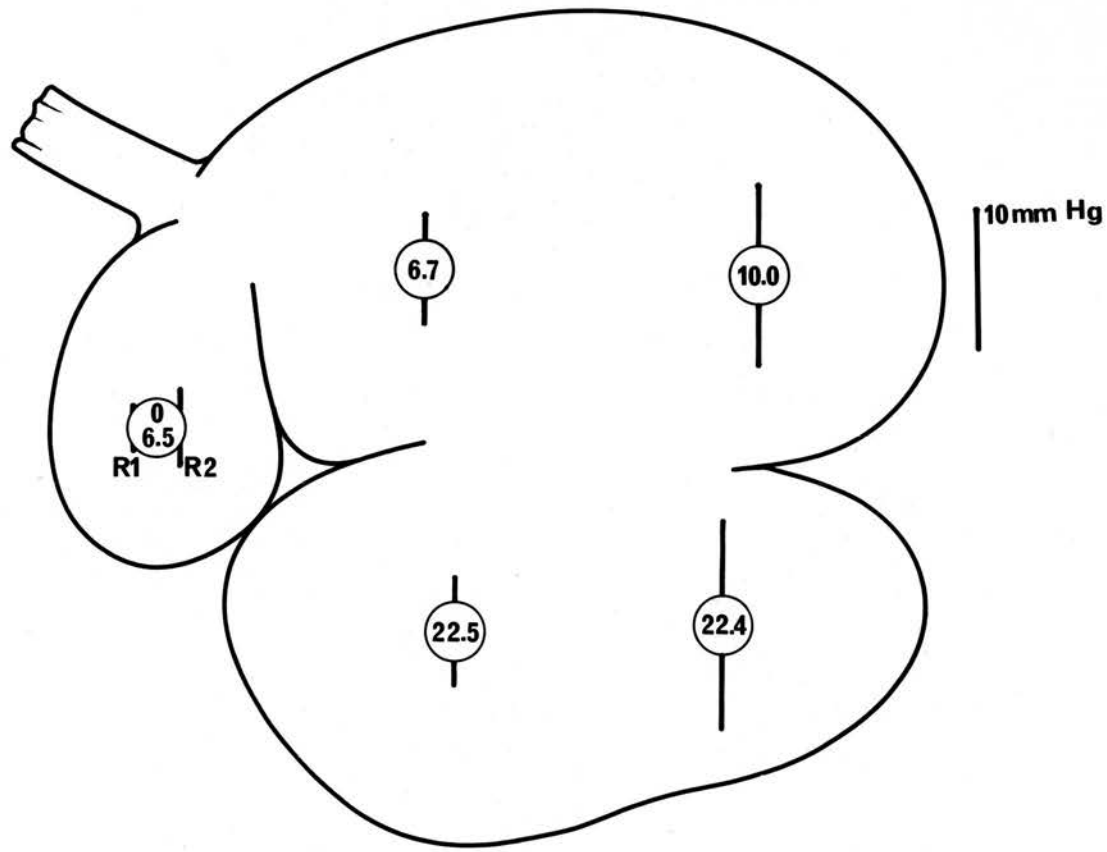
anterior and posterior parts of the ventral sac of the rumen. These balloons, measuring 3 cm. in diameter, were connected by stiff polythene tubing to Marey tambours, the writing points of which were arranged to give a record of pressure events at each of the positions mentioned. (The techniques for fistulation and recording are presented in greater detail in section 2). It will be seen from Fig. 2 that the peak pressure changes detected at these different points occurred at different times and showed marked differences in amplitude, even though the balloons were only a few cm. apart (about 8 cm. in the case of balloons in the anterior dorsal sac of the rumen and the reticulum). One is therefore quite justified in assuming that pressure events within the rumino-reticulum are localised, and vary with the position of the sensing head.

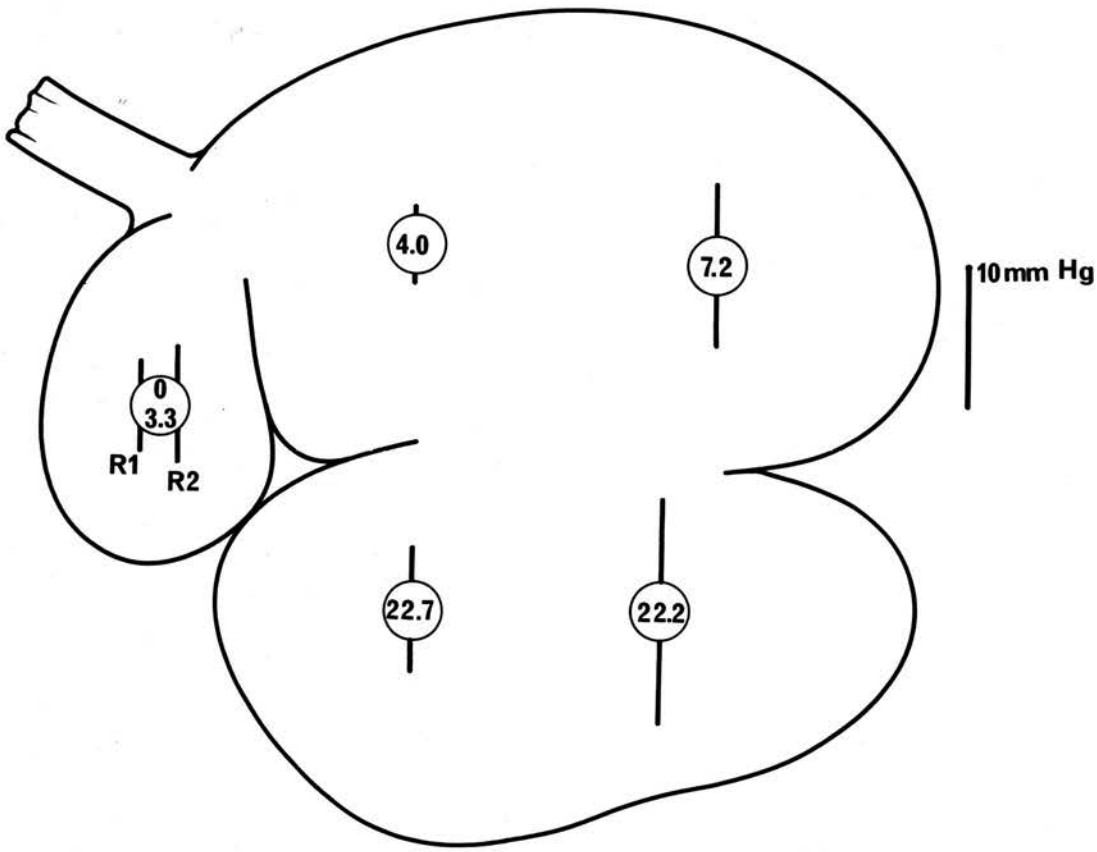
If relatively large balloons are used (of capacity 1 L or more), and are inflated with say 100 ml. air, the rises in pressure recorded are of similar amplitude to those seen when smaller balloons were used, but pressure events may last up to 25% longer, and fluctuations associated with respiration appear relatively much larger.

Theoretically, two systems for recording pressure changes are possible - isometric and isotonic - depending on the design of the sensing head. However, in the case of small balloon systems inserted into various compartments of the rumen, a special case arises. As the volume of the balloon relative to that of the rumen, is very small, it can for the purposes of this discussion, be effectively ignored. In a complex, 3-phase system of solid, liquid and gas, contained in an organ such as the rumino-reticulum, in the walls of which contractile events are relatively localised at any one time, it is evidently quite futile to pursue such a classification, unless it is applied only to the sensing head,

Fig. 2. Pressure events recorded from different points within the rumino-reticulum during primary rumination (upper) and non-rumination (lower) contraction cycles. Data were obtained from kymograph tracings from a conscious adult sheep, starved for 7 hours and carrying two fistulae of the dorsal rumen sac, 14 cm. apart. Each value presented is the mean of measurements taken from 10 successive contraction cycles. Amplitudes of pressure are indicated by the vertical lines (calibration on the right), and the times of peaks of pressure rises (in secs.) are contained in the circles.

On the original records, changes in pressure of 0.2 mm. Hg. could be distinguished, but there is no evidence, at this sensitivity, for the passive transmission of pressure events at least over distances of 8 cm.


10mm Hg
6.7
10.0
0
6.5
R1
R2
22.5
22.4


10mm Hg
4.0
7.2
0
3.3
R1
R2
22.7
22.2

without regard for the physiological components of the system.

A somewhat special case could be established in the reticulum, if this organ were to be totally filled with a sensing device, such as a large, inflated balloon. If the volume of this organ is taken to be 1 L (from data presented by Sisson & Grossman, 1958), then it might be argued that a balloon of this volume, lying within the lumen of this viscus, could be used in either an isotonic or an isometric sense. This, unfortunately, does not appear to be the case. If the appropriate volume of fluid is added to a balloon lying within the lumen of the reticulum, it is found that motility in the organ may be totally inhibited. Moreover, the addition of only 200 ml. of warm water to a balloon lying inside the reticulum is sufficient to induce rhythmic motility of the organ, a finding which implies that the consequences of this procedure can be detected by the animal; in the absence of evidence for sufficiently sensitive volume receptors, within the rumino-reticulum, it seems likely that the wall of the reticulum is being stretched. Until a more complete analysis of the physical conditions within the organ becomes available, it would seem more prudent to avoid use of the terms 'isotonic' and isometric' as applied to recording conditions within the rumino-reticulum.

Since the events recorded by a pressure sensing device are secondary to changes in tension in the wall of the stomach, it is clear that fluctuations in pressure must be transmitted over a finite distance before they are able to affect the probe. During this time, the mechanical event is being transmitted through a liquid phase, through which is irregularly disposed a solid phase (consisting of food particles which vary in nature and length), imparting a viscous component to the dynamic system. Moreover, contraction of the stomach is accompanied by

movement of the gastric contents, indicating that the pressure does not rise simultaneously and equally throughout the organ. The existence under such conditions of an appreciable viscous component is of great importance, because this will act as a damping element, and should be taken into account when determining the frequency response of the system. It is probably this factor which is largely responsible for the failure to record high frequency phenomena from the interior of the rumino-reticulum, even when critically damped, high frequency-response catheter systems are used (see, e.g., Dzuik, Fashingbauer & Idstrom, 1963).

A further complication is that pressure at a given point depends on events in a finite area of the stomach wall, which increases as the square of the distance from the wall; the use of balloons as sensing devices exaggerates this effect. This may be the reason why Bell (1958) reports pressure rises of up to 65 cm. of water from the reticulum of the goat, using a polythene tube passed into the reticulum via the oesophagus in conjunction with a capacitance monometer; pressures recorded in the present work (up to 15 mm. Hg.) are typical of those measured with balloon systems. Routine use of fine-bore catheters, however, would demand precise location of the tip in relation to the stomach wall, if recordings taken on different occasions were to be comparable. If this arrangement were to be adopted, the system would approach that described under sub-section 2.

It follows, from the preceding discussion, that the high frequency components which might theoretically be expected to occur within the lumen of the forestomach are markedly attentuated, and in consequence, it is unnecessary to prepare elaborate, high frequency response systems to record pressure fluctuations within this viscus. Providing rigid connecting tubes of minimum length are used to couple the transducer to the sensing

head, it was found that the pressure events recorded by a Marey tambour writing on smoked kymograph paper (frequency response 1.5 to 2 c.p.s.) showed a very close similarity to those recorded using an air transmission line with a frequency response of 20 c.p.s.

The experimental work described in this thesis has demanded the use of a consistent and reliable method for routine recording of the rumino-reticular motility. This has been accomplished by recording changes in pressure from within the forestomach, using a variety of methods which are described in greater detail in the relevant sections. The records obtained will be referred to as indicating gastric contraction, in accordance with general procedure, although it must be realised that all the reservations laid down in 5 above must be applied to the data obtained.

EXPERIMENTAL OBJECTIVES

The present investigations were designed to provide further information on the nature of the reticulo-ruminal motility centre. Observations were also made on some of the factors involved in the genesis of periodical bursts of activity which pass down the vagi and result in a contraction cycle of the rumino-reticulum.

In the first instance, the method chosen to demonstrate the central origin of the efferent fibres was a histological one; after section of nerves supplying the stomach, the positions of cells showing chromatolysis were demonstrated. During these experiments the opportunity was taken to investigate the transitional motility patterns as rumino-reticular contractions were re-established. It was hoped that this would assist evaluation of the evidence for a 'subcentre' within the gastric wall, co-ordinating motility of the rumino-reticulum. Results of these studies led to further investigations into the innervation of the various gastric compartments. All information obtained in relation to this problem will be presented in section 3.

When attempts were made to confirm the localisation of the dorsal nucleus of the vagus by point electrical stimulation, it was found that inhibitory influences in the region were very pronounced, and these were accordingly studied in some detail. Finally, attempts were made to record activity related to gastric motility from single units within the dorsal nucleus of the vagus. Results from these experiments are described in section 3.

SECTION 2

METHODS

Experimental Animals

The experiments to be described were carried out on Blackface or Blackface cross ewes and wethers aged 9 to 24 months, weighing 25 to 35 Kgm. Animals were kept at pasture until 2 to 6 weeks before they were required, and were then transferred in batches up to 6 to an indoor pen measuring approximately 5 x 4 metres; hay and water were provided ad lib. This ration was supplemented with crushed oats when the quality of the hay was poor. No attempt was made to starve individual animals before acute experiments, as preliminary tests suggested that it was more difficult to establish rhythmical motility under experimental conditions following this procedure.

When animals were permitted to recover (as in the experiments to determine the effects of transection of a thoracic vagal trunk), they were haltered to restrict movement, until the effects of the anaesthetic were no longer apparent (usually 1 to 2 hr.). Sheep were then transferred to a metabolism cage measuring 1 x 0.5 metre, provided with facilities for giving food and water. For the first 3 days following surgery, 400 g. rolled oats were given, and a constant supply of clean water and good quality fresh hay was available ad lib. After 3 days, experimental animals were returned to the stock pen, and received thereafter a daily allowance of 300 g. crushed oats immediately after each recording session. On the day following surgery each subject received two intramuscular injections of antibiotic (0.5 M units procaine Penicillin with 0.5 G dihydrostreptomycin - brand 'Strypen' M & B) at a 10 hr. interval, and subsequently received one such injection for the following two days.

Using this regime, body weight fell by about 1 Kgm. over the 3 week experimental period, and no animals over 9 months old died or had to be destroyed during this time. Four animals under 9 months old (6 to 9 months) failed to regain their appetite following vagotomy, and all of these were destroyed. On post-mortem, these animals were dehydrated subcutaneously, but there was a little clear serous fluid in all three coelomic cavities the heart, liver and kidneys showed moderately extensive fatty change. The clinical and pathological features resembled those seen in metabolic disorders of the ketotic types and one additional animal which developed the early clinical signs showed a good recovery following two successive daily injections of 25 mg. prednisalone acetate (brand 'Deltastab' - Boots). Data from this animal were treated separately.

Anatomical and Histological Investigation of the Nerve Supplies to the Stomach.

The vagal innervation of the ovine stomach was examined in a series of six Scottish Blackface sheep, about 1 year old, by gross dissection. Fresh material was obtained from the slaughter house, or from experimental animals killed for other purposes; the nerves were most easily identified and traced if the organ was removed from the thin sheep when there was little fat to obscure them; fresh material was superior in this respect to formalin or alcohol fixed material.

Dorsal and ventral vagal trunks were identified in their relative positions at the cardia, and were then followed backward over the stomach wall and omentum using dissecting spectacles to identify the finer branches. With care, branches of 0.2 to 0.4 mm. diameter could be followed in this way. Saturated aqueous picric acid solution was applied with a swab of cotton wool to assist the differentiation of nerve trunks from blood- and lymphatic - vessels. Usually the ramifications of the dorsal trunk were followed first, and when they had been completely displayed and drawn, they were dissected free of the stomach and the ventral trunk was then traced; in this way, confusion between the two nerves was avoided.

On three occasions, short lengths of nerve (about 1 to 2 cm.) were removed from various points on the stomach for histological investigation. Such material was labelled as soon as it was removed, was carefully washed in saline, and then stretched out on a piece of dry filter paper, lining the base of a petri-dish. Five ml. of fresh 1% osmic acid solution in 0.9% saline were then carefully poured onto the filter paper in such a way as to avoid dislodging the specimens - this volume of solution was completely absorbed by the filter paper, so that fixation and staining was the result of diffusion of the reagent. The lid

of the petri-dish was replaced, and the material was left in the dark for 24 to 48 hr., when it was removed and thoroughly washed in saline. Dehydration was achieved by prolonged immersion in low grades of alcohol (24 hr. in each of 30%, 50% and 70% alcohol) followed by treatment of 1 hr. with 90%, and then two changes of absolute alcohol. After clearing for 1 hr. in benzene, tissues were embedded in paraffin wax (M.P.49^{o}C) using a vacuum embedding bath, blocked, and transverse sections cut at 4μ on a Reichert rotary microtome (Model OmS). Serial sections were permanently mounted in D.P.X.

The techniques adopted for counting and preparing histograms of the myelinated fibres in the various nerve trunks, was to photograph representative complete sections onto Ilford R20 panchromatic plate, using a Zeiss standard universal microscope with a Zeiss 10 x Planapo objective and with an oil film between the condenser and the base of the slides. Focusing was performed with the help of a magnifying glass placed over a ground glass screen. Plates were developed in Demancy's D.V.P. developer for 3 min., fixed, washed and dried. Enlargements were prepared from these plates to provide an overall magnification of 1000 x, on Ilford B21P Bromide paper, and these were edited to provide a complete section of each nerve, on which the myelinated axons were clearly visible. Counting was performed by measuring mean external axon diameter with an engineer's rule, recording each measurement on a graph, and pricking through the print with a pin to prevent any axon from being missed or counted twice. With the exception of the dorsal vagal trunk, two sections from each series were treated in this way, and if the final counts and histograms differed in any respect by more than 5%, the data were discarded and the count repeated.

Efferent Electrical Stimulation of the Thoracic Vagal Trunks

Five experiments were performed in which the peripheral stump of the transected dorsal or ventral thoracic vagal trunk was electrically stimulated using a totally enclosed electrode of the type described by Garry & Wishart (1951). Blackface wethers weighing 25 to 30 Kgm. were narcotised by slow intravenous infusion of 1% chloralose solution (50 mg. per Kilo) at 80°C, to give a deep level of sedation. A tracheal cannula was then inserted, so that positive pressure ventilation could be provided from a Palmer respiration pump as required. With animals on their right side, the fleece was then clipped from the entire left body wall, and a 15 to 20 cm. long incision made into the abdomen, about 3 cm. posterior to and parallel to the costal arch. Air-filled balloons attached to the end of rigid polythene tubing were then inserted into the reticulum, dorsal rumen sac and ventral rumen sac via small incisions in the gastric wall, closed with purse-string sutures, and pressures within the system were continually monitored via Marey tambours on smoked kymograph paper. The frequency response of the recording system, with the balloons 'in situ', was about 2 to 3 per sec.

After resecting the skin from the left thoracic wall, the middle two quarters of ribs 8 to 12 inclusive was resected. Artificial ventilation was established as the thorax was opened. The dorsal and ventral trunks of the vagus were then located and sectioned posterior to the anastomosis between them, and the stimulating electrode placed around the trunk to be studied. Throughout the duration of the experiment, evaporation from the exposed surfaces was prevented by the liberal use of liquid paraffin, and the exposed lungs were protected with paper towels soaked in warm saline.

Five second trains of biphasic pulses, 5 msec. in duration,

were delivered to the nerve from a Grass SD5 fully isolated stimulator, varying the voltage and frequency of stimulation systematically up to 5 V and 80/sec. respectively; all observations were repeated before the electrode was placed over the other vagal trunk, and the investigation repeated.

SURGICAL TECHNIQUES

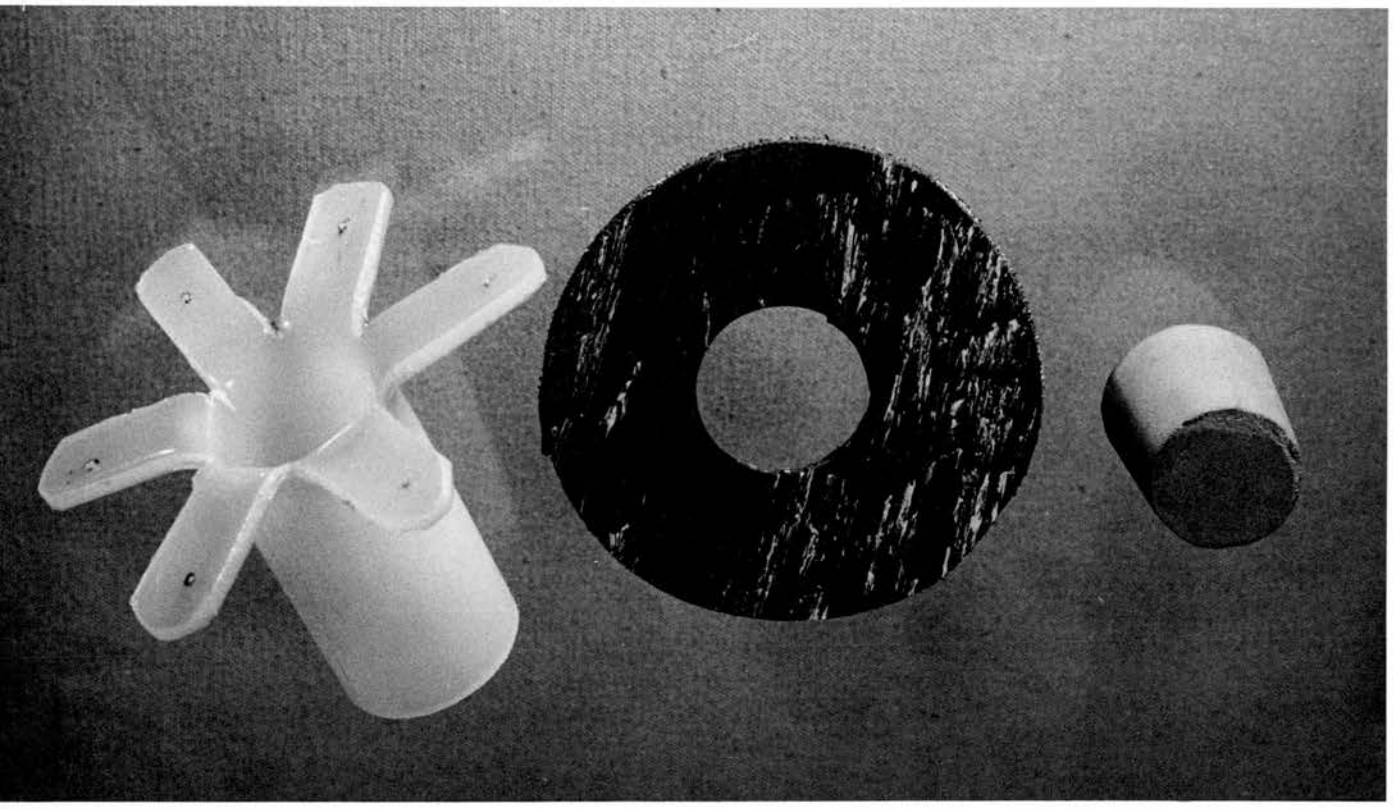
Rumen Cannulation

Rumen cannulae of the type shown in Fig. 3 were used throughout.

This cannula was developed by Mr. B.F. Leek in the Department of Veterinary Physiology at the Royal (Dick) School of Veterinary Studies, for chronic use in sheep. The type employed here was made from polythene tubing 3 cm. in internal diameter, and wall 3 mm. thick, cut into 10 cm. lengths. Three longitudinal cuts, 4 cm. deep, were made across the diameter of the tubing at one end; the lugs thus formed were bent outward at right angles under heat. About 1 cm. from the end of each lug a 1.5 mm. hole was bored through which thread could be passed to secure the ends during insertion. The washer was cut from 3 mm. thick reinforced rubber matting, so that the outer diameter was about 10 cm., and the inner diameter 3.6 cm., to fit snugly over the barrel of the cannula.

The 6 polythene lugs on the cannula are drawn together and tied with thick thread, and the entire assembly is sterilised in 0.02% aqueous solution of chlorhexidine (brand 'Hibitane' I.C.I.). Anaesthesia was induced with Halothane (brand Fluothane I.C.I.) administered from a shaped face-mask; Halothane was also used for maintenance, delivered to an endo-tracheal tube, to-and-fro circuit. An area in the left paralumber fossa was shaved, scrubbed and disinfected with 1% chlorhexidine. Using full aseptic precautions, four interrupted sutures were inserted through the skin and underlying rumen wall to isolate a circle 8 cm. in diameter. A stab incision into the rumen was made at the centre of this, and enlarged just sufficiently to accommodate the cannula, the tied end of which was then inserted into the

Fig. 3. The cannula assembly used for chronic implantation into the mid-dorsal sac of the rumen. In use, the lugs of the cannula are sprung together with thread to form a cone which is pushed into the rumen via a stab incision in the left flank. The thread is now cut so that the lugs splay outward and rest against the inner rumen wall. The rubber washer (centre) is pushed over the barrel of the cannula and secured with zinc oxide adhesive tape. A bung is used to close the open end of the cannula.



incision until it could be seen to have entered the rumen. Ligatures securing the lugs were now cut with a sharp scalpel, so that the lugs splayed outwards, resting against the inner surface of the rumen wall. Finally the rubber washer was slipped over the cannula to lie flush against the skin, and secured by wrapping zinc oxide adhesive plaster around the barrel of the cannula; a rubber bung was used to close the cannula and administration of anaesthetic was discontinued. With practice, the entire operation could be completed in 30 min.

Transection of thoracic vagal trunks.

Approximately 1 week after cannulation of the mid-dorsal sac of the rumen, anaesthesia was again induced with Halothane, a cuffed endo-tracheal tube inserted, and a to-and-fro anaesthetic circuit established. The skin behind the left shoulder extending back to the costal arch was closely shaved, scrubbed and disinfected with 1% Chlorhexidine. Using full aseptic precautions, the skin over the tenth rib was incised, and approximately 9 cm. of the mid portion of the tenth rib was removed (attempts to leave the periosteum intact usually proved unsuccessful). Positive pressure respiration was now applied with a Palmer respiration pump (stroke volume about 350 to 450 ml., rate 25/ min.), the pleura was opened, and the oesophagus located. In cases of difficulty, the passing of a thick-walled rubber tube into the oesophagus greatly facilitated this procedure. The dorsal or ventral trunk of the vagus was then easily located running immediately above, or about 1 cm. below the oesophagus respectively. A pair of rat-toothed forceps was used to tear the mediastinum from over the nerve trunk, and the latter was then picked up on a nerve-hook, grasped with two pairs of artery forceps, and then torn completely across.

No attempt was made to re-constitute the peritoneum. Penicillin (0.5 M units) and dihydrostreptomycin (0.5 G) (brand 'Strypen' - M & B) was sprayed into the thoracic cavity using a syringe and fine hypodermic needle, and the thoracic wall was closed using three layers of continuous sutures in No. 2 BPC nylon, and a single row in interrupted horizontal mattress sutures to close the skin. During final closure of the thoracic cavity, the lungs were fully distended by blowing down the endo-tracheal tube, so that as much air as possible was removed from the thorax. Administration of anaesthetic was then discontinued, and the animals allowed to recover. Vagotomy was usually completed within 1 hr.

No deaths were directly attributable to this operation.

The 'Routine' Recording of Pressure Events from the Forestomach

Three days after the rumen cannula had been placed in position, animals were trained to stand quietly in a metabolism cage while they were being handled, and while balloons were inserted via the cannula into different gastric compartments. Sheep usually stood quietly during such manipulations after about 2 days, and at this stage, recording was commenced. Small air-filled balloons (5 cm. diameter) were attached to copper tubing 3 mm. in external diameter (internal diameter 2 mm.) which was bent to allow insertion of balloons into the different parts of the forestomach. These balloons were then inserted through the cannula so as to occupy positions in the reticulum, and mid-dorsal and mid-ventral sacs of the rumen; they were fixed in their relative positions by packing tightly within the cannula with cotton wool. By using the same sensing devices for each experimental period, balloons could be located within the forestomach at similar positions on successive days. All recording was carried out using Marey tambours and a kymograph, with air transmission from the sensing balloon. Balloons placed over the left jaw (beneath a halter) and left oesophageal groove, coupled similarly via polythene tubing to Marey tambours, recorded chewing and swallowing events respectively. A 30 sec. time trace was added routinely.

Except on the day following nerve section, recording was carried out over a period of 30 to 45 min. each day, at a comparable time, in case diurnal fluctuation should complicate interpretation. In one experiment, balloons were kept in position in the reticulum and rumen during section of the dorsal vagal trunk, to determine the immediate effect on motility. Immediately following each period of recording, a full clinical examination was given, and the allowance of crushed oats

(300 or 400 gm.) was given, before returning the sheep to the stock pen.

Data for each day have been taken from the recordings of 10 successive contraction cycles of the forestomach, comprising measurements for amplitude, duration and the temporal relation to the beginning of the motility cycle (i.e. the first indication of the first contraction of the reticulum) for each gastric record, when the animal was not ruminating, and, where possible, also when it was ruminating. The figures obtained in this way relating to each parameter, have been averaged, and the mean values and standard deviation calculated by punched-tape presentation to an Atlas computer (the programme used is presented in the appendix).

RESULTS

The distribution of the dorsal and ventral vagal trunks over the stomach is shown in Figs. 4 and 5. Although the position of the anastomosis between dorsal and ventral trunks anterior to the disphragm showed a considerable degree of variation in the six specimens examined, the course of nerves over the stomach was very constant, varying largely in the size of individual branches rather than the routes they took. It was not possible to trace fibres beyond the pylorus of the abomasum onto the duodenum, although fine connecting branches could be seen passing to the sympathetic ganglia as indicated. The chief findings may be summarised thus:-

The dorsal vagus, running closely applied to the upper surface of the oesophagus, divides at the cardia and sends

1. a number of twigs to the anterior aspect of the reticulum;
2. a long nerve trunk running along the abomasal mesentary; closely applied to the abomasum, and supplying this latter. It also sends a few fine twigs to the omasum, although on two occasions this was supplied by a separate trunk, as shown in Fig. 4.
3. The right ruminal nerve (adopting Habel's terminology) separates here, and passing back in the right longitudinal rumen furrow supplies branches to the dorsal and ventral sacs of the rumen.
4. A very fine branch passes downward, through the anterior groove (or furrow) and ramifies over the right face of the rumen. Grossly, the area supplied by this 'left ruminal nerve' is very restricted; it could not always be traced right through the groove.

Fig. 4. The distribution of the dorsal vagal nerve trunk over the ovine stomach, as revealed by gross dissection. Adopting Habel's terminology, the major branches are:-

1. twigs to the reticulum
2. Abomasal nerve
3. Right ruminal
4. Left ruminal (double in this particular case)
5. Dorsal ruminal (not described by Habel)

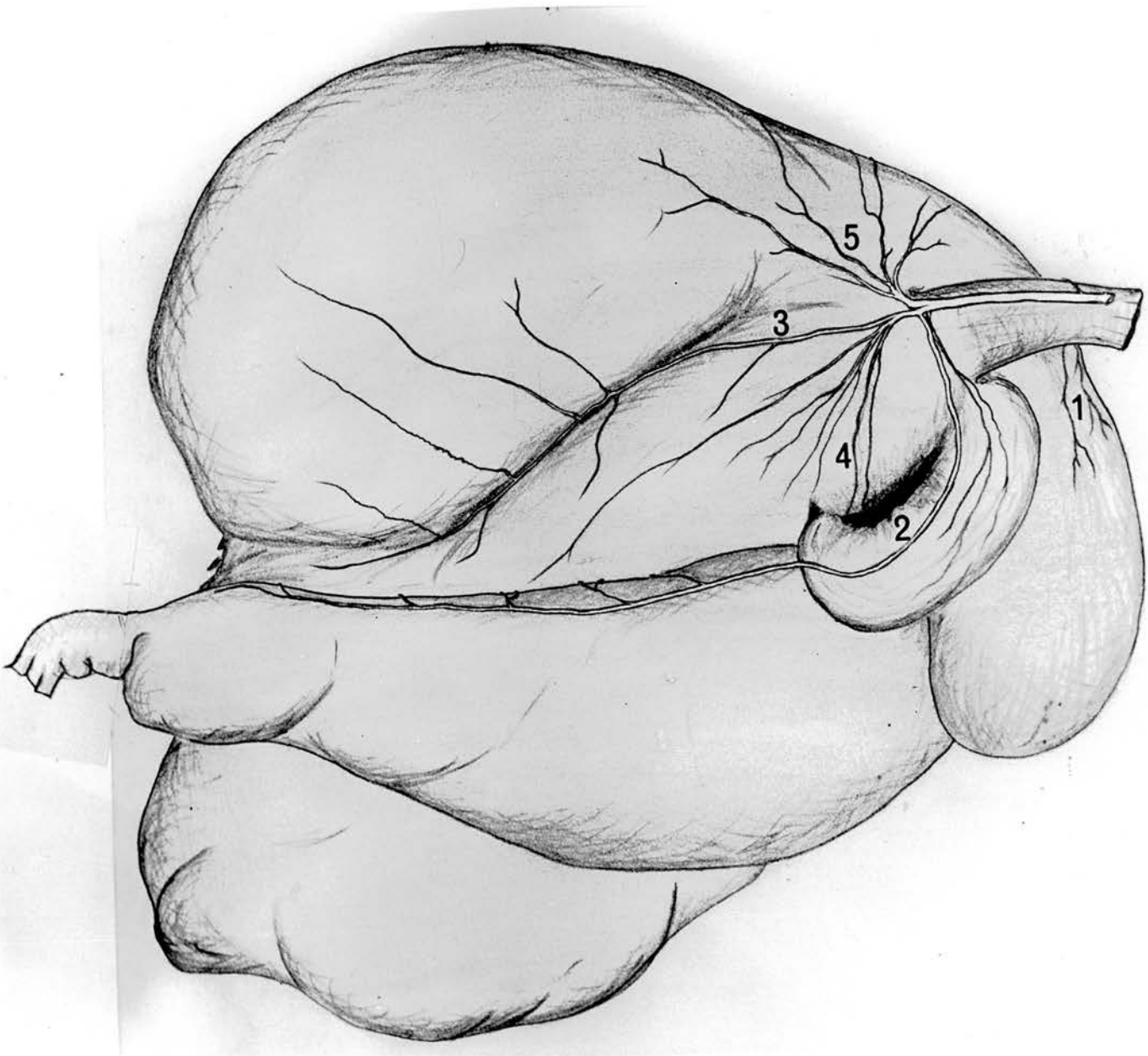
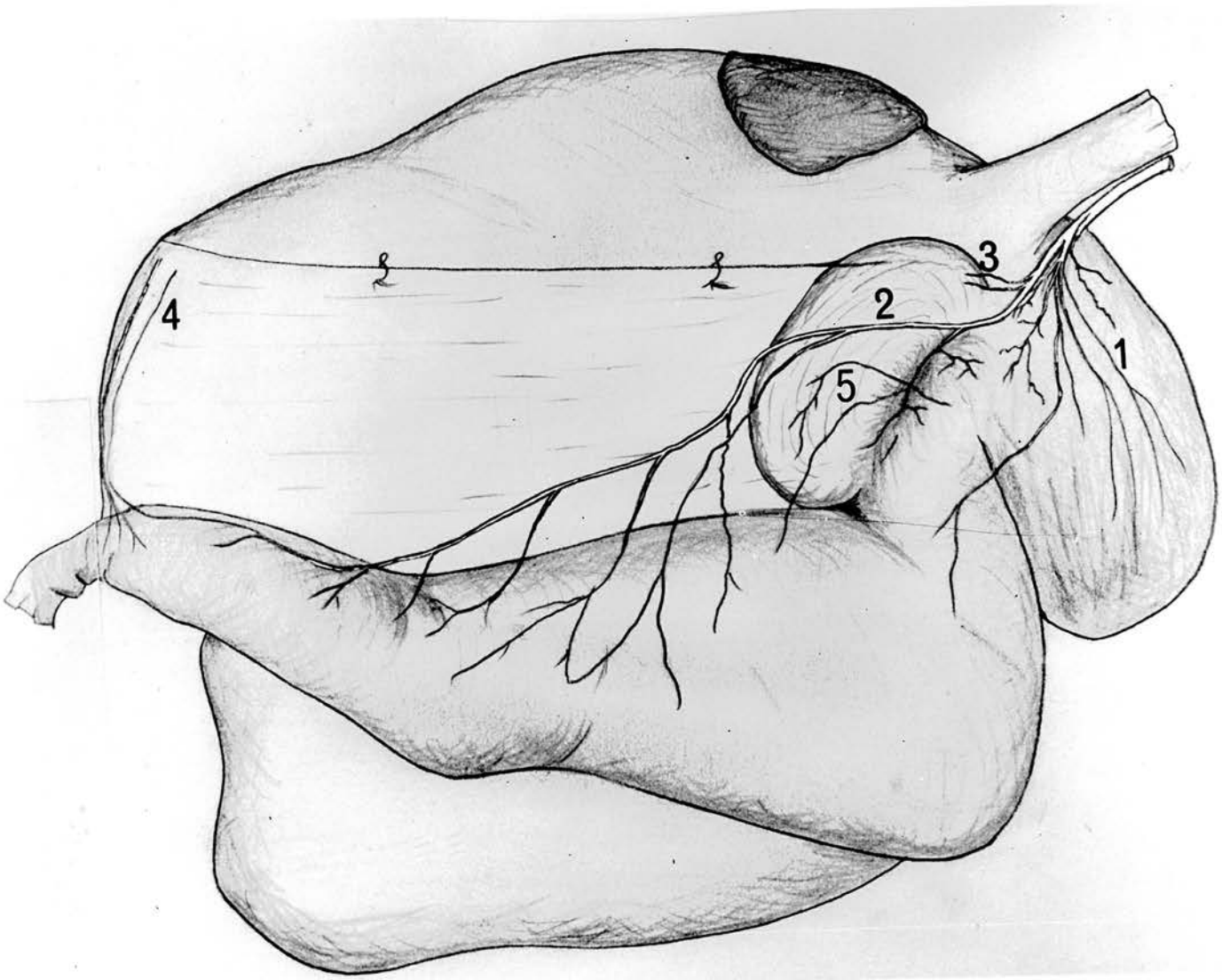

5
3
1
4
2

Fig. 5. The distribution of the ventral vagal nerve trunk over the ovine stomach, as revealed by gross dissection. Adopting Habel's terminology, the major branches are:-

1. twigs to the reticulum
2. abomasal nerve
3. branches running toward the liver
4. anastomosis with a twig from the coeliac ganglion
5. strands supplying the omasum.


4
3
2
1
5

5. A number of fibres pass dorsally over the anterior part of the dorsal sac of the rumen and supply here a limited area of the left face of the rumen.

The ventral vagus runs some distance below the oesophagus, in the mediastinum, and has a rather more limited distribution from the cardia, supplying:-

1. Large numbers of fibres to the right and anterior faces of the reticulum.
2. A long nerve, running fairly high up in the mesentary, along the lesser curvature of the abomasum which it supplies, and also sends a few strands to the omasum.
3. Two small strands also separate at the cardia and run toward the liver - these were not traced to their termination, but Habel describes their course to the sympathetic ganglion.
4. A delicate nerve from the region of the caeliaco - mesenteric ganglion makes an anastomosis with the terminal end of the abomasal branch of the ventral vagus.

Although gross anatomy failed to indicate any innervation of the rumen by the ventral vagal trunk, finer branches could pass from this nerve to the rumen, and supply it with motor nerve fibres. If this is so, electrical stimulation of the peripheral end of the transected ventral vagal trunk would cause contraction of the rumen.

The responses to electrical stimulation for periods of 5 sec. at different frequencies and voltages of the dorsal and ventral trunks of the vagus are shown in Figs. 6 and 7. Sensitivity of the different pressure traces was adjusted so that a pressure change of 0.1 mm. Hg. caused a deflection of 1 mm. on the kymograph trace; allowing for variation in baseline level associated with respiration, etc., this procedure would allow

Fig. 6. The mechanical response of the ovine forestomach to electrical stimulation of the dorsal vagal trunk, 5 cm. anterior to the cardiac arifice. The positions of recording balloons are indicated in the insert, and the symbols used on the graphs correspond to these.

Stimuli were delivered from a fully isolated Grass SD5 stimulator as 5 msec pulses at 4/sec for 3 secs. Responses are shown at increasing voltages.

Motor fibres innervating the reticulum have a lower threshold than those supplying the rumen, but both gastric compartments contract strongly as the intensity of stimulation is increased. The maximum amplitude of contraction under these conditions, from any of the three points, was 7 mm. Hg. Note how little the time relationships of reticular contraction vary as the strength of stimulation is altered.

Each point on the graph is the mean value from 3 successive determinations. Similar traces were obtained from each sheep studied.

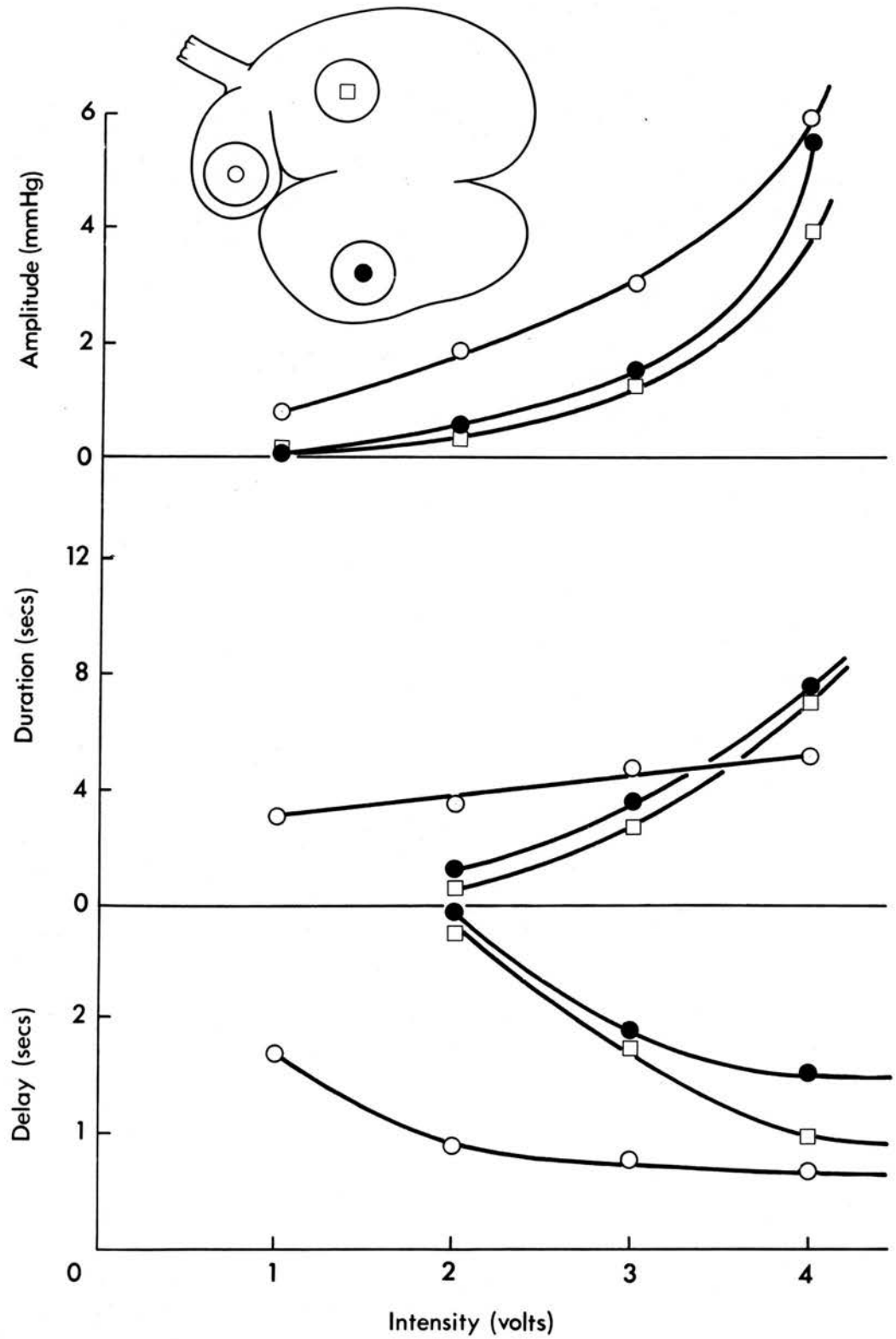
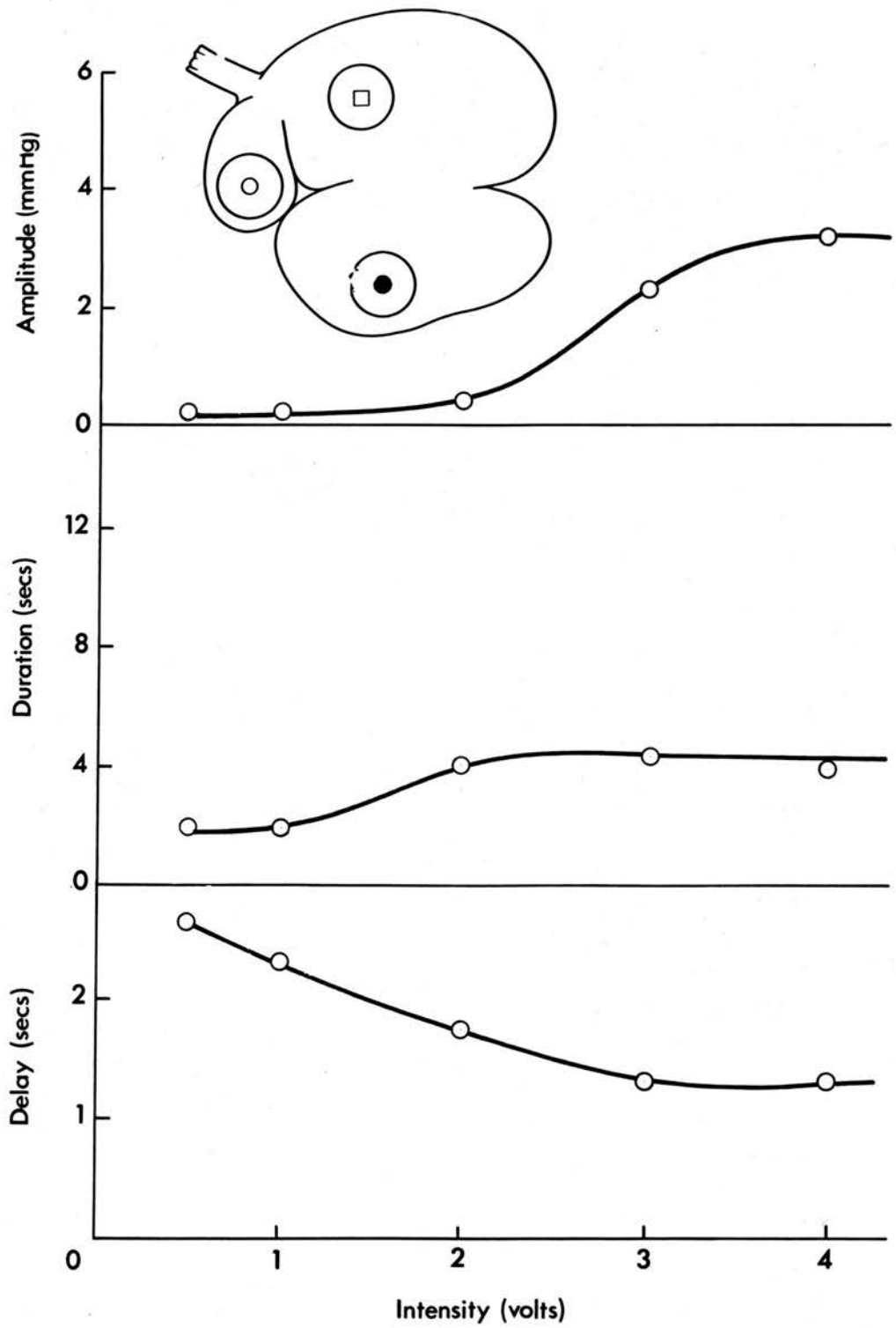

Amplitude (mmHg)
6
4
2
0
Duration (secs)
12
8
4
0
Delay (secs)
2
1
0
1
2
3
4
Intensity (volts)

Fig. 7. The mechanical response of the ovine forestomach to electrical stimulation of the ventral vagal trunk, 5 cm. anterior to the cardiac orifice. The positions of recording balloons are indicated in the insert, and the symbols used on the graphs correspond to these.

Stimuli were delivered from a fully isolated Grass SD5 stimulator as 5 msec pulses at 4/sec for 3 secs. Responses are shown at increasing voltages.

Contraction of the rumen was never detected, even when the intensity of electrical stimulation was increased to 6 V. The shortest interval between stimulation and the onset of contraction of the reticulum is about twice that for stimulation of the dorsal vagal trunk at the corresponding position.

Each point on the graph is the mean value for 3 successive determinations. Similar traces were obtained from each sheep studied.


Amplitude (mmHg)
6
4
2
0
Duration (secs)
12
8
4
0
Delay (secs)
2
1
0
1
2
3
4
Intensity (volts)

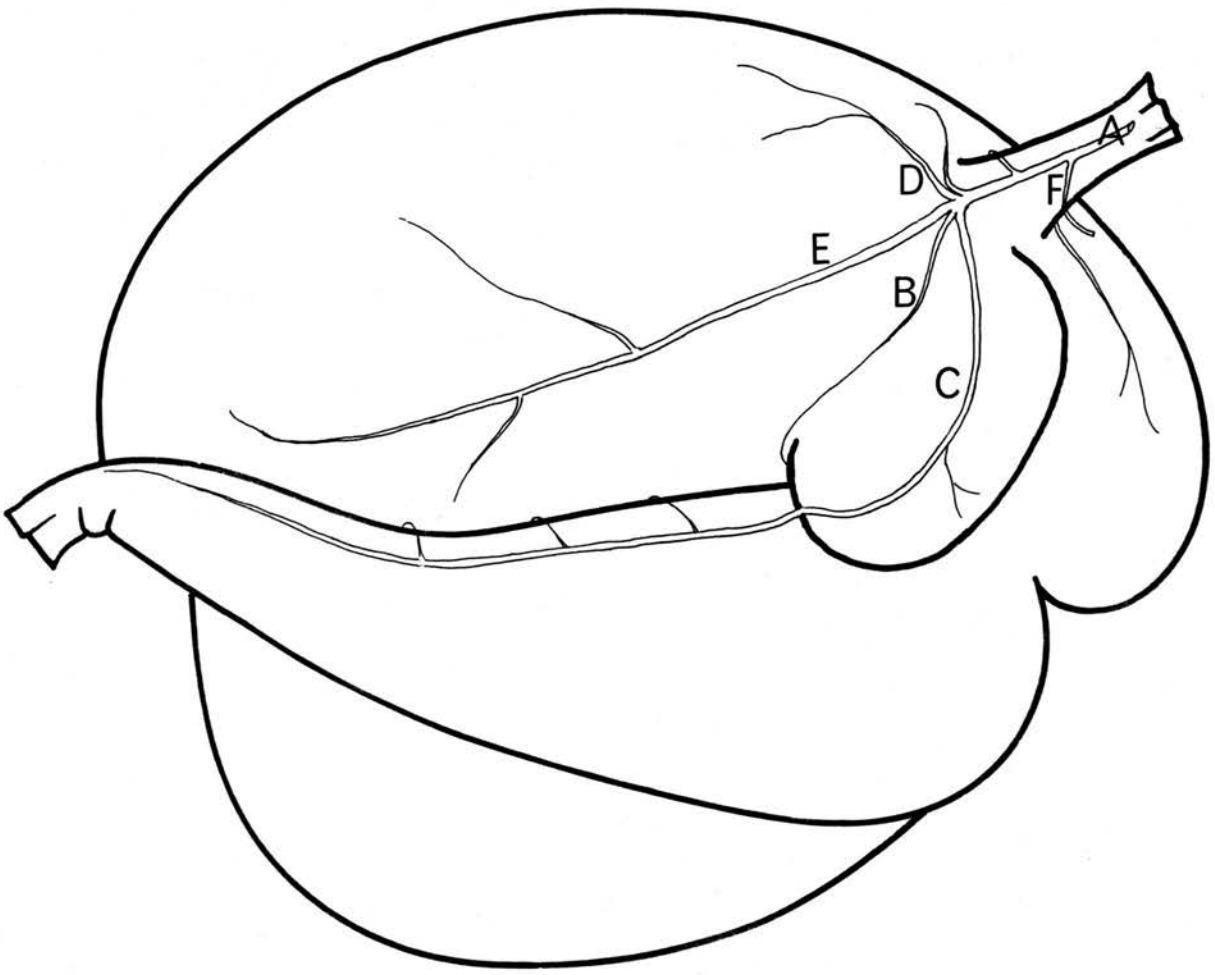
pressure rises of 0.2 mm. Hg. to be seen in the rumen, or 0.5 mm. Hg. in the reticulum.

Stimulation of the peripheral stump of the transected dorsal vagus elicited pressure rises of up to 1 mm. Hg. from the reticulum without corresponding increases in pressure elsewhere; at higher voltages (over 1.0 V at 4 c.p.s.) contraction of dorsal and ventral sacs of the rumen occurred. Electrical stimulation of the ventral abdominal vagus, at strengths of 4 V or more, failed to elicit any pressure change in the rumen, although the reticulum contracted strongly (see Fig. 7). Direct observation of the left rumen wall through the laparotomy incision confirmed the absence of contraction of the rumen wall on electrical stimulation of the ventral vagal trunk.

The data obtained from the histological investigation of gastric innervation in two sheep are presented in Figs. 8, 9 and 10. The dorsal thoracic vagus contains approximately $2\frac{1}{2}$ times more myelinated axons than the ventral thoracic vagus, although the histograms showing fibre size are very similar. Most of the myelinated fibres contained in the anastomotic branch between dorsal and ventral vagi are of relatively very large diameter (mean 4 to 5μ), and this nerve is contained in a thick sheath of fat; grossly, it appears much larger than it actually is. The number of myelinated fibres exchanged at this point is only about 1500 to 2000, and there is little of the cross-sectional area unoccupied by myelinated axons.

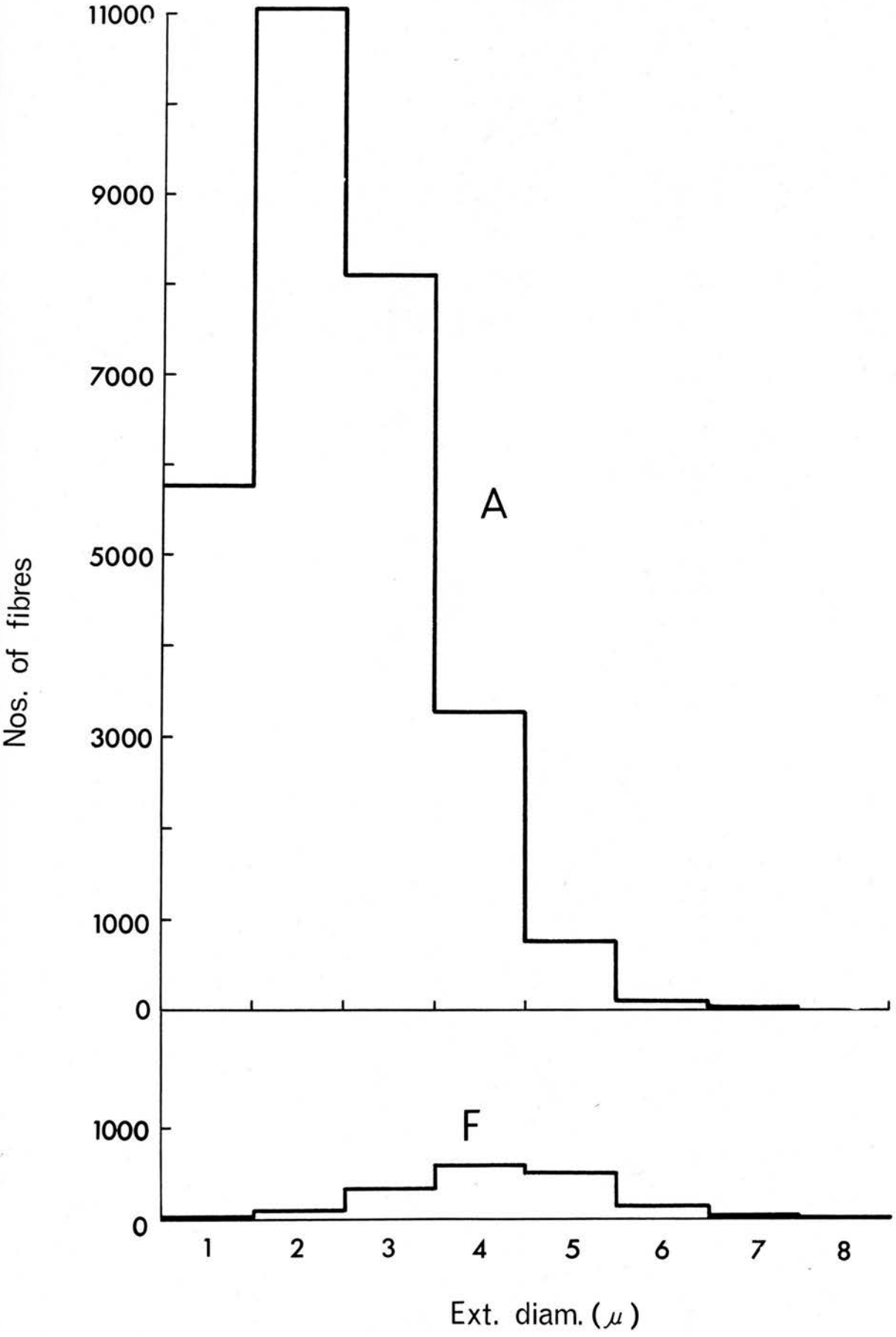
There is a difference in the histograms between nerves supplying different parts of the stomach, although the difference between corresponding branches from the dorsal and ventral vagal trunks is much less marked. Thus, the myelinated fibres which innervate the abomasum appear to be largely of small diameter (1 to 2μ); those supplying the omasum and the region of the

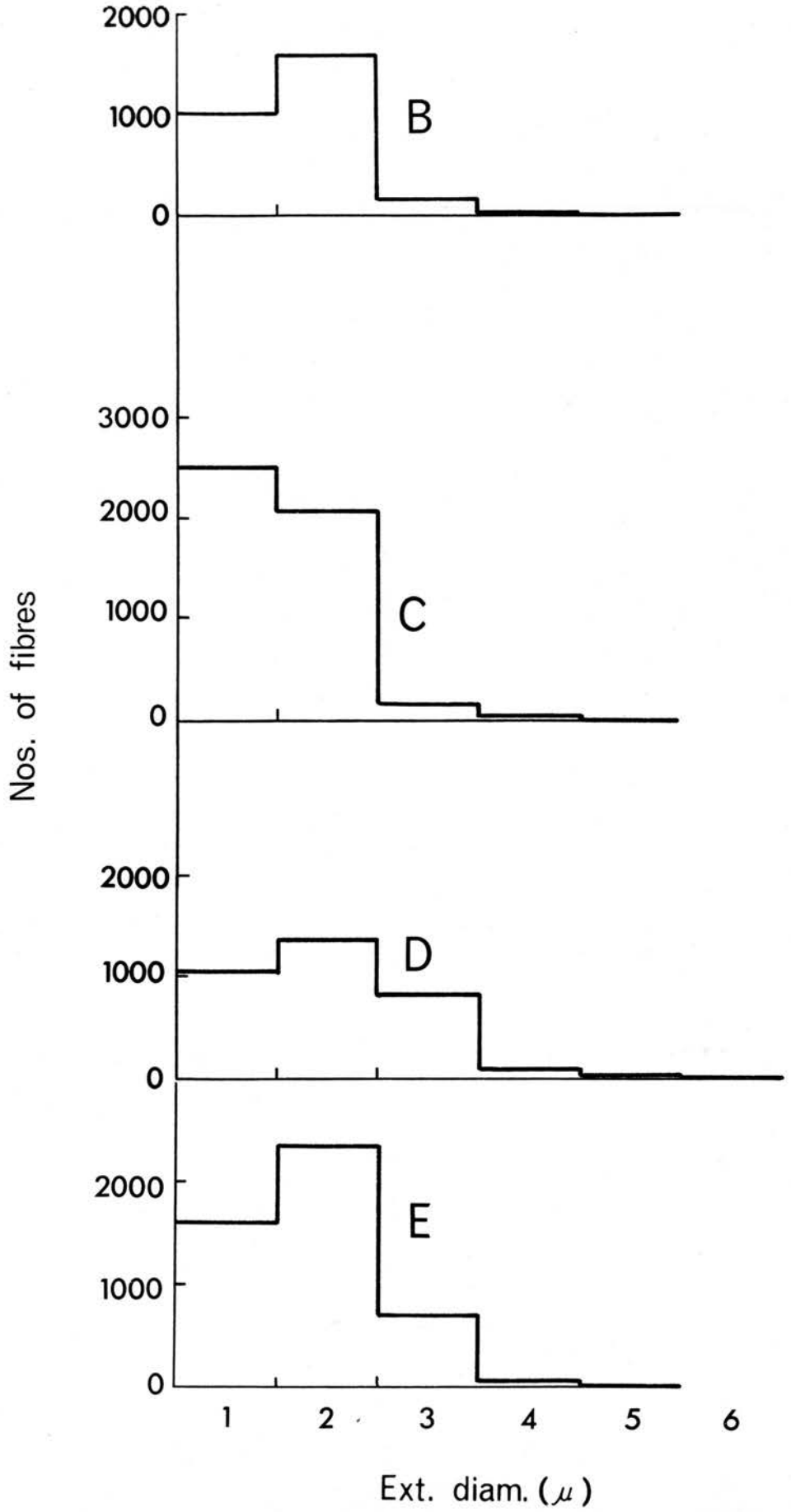
Dorsal vagus



Figs. 8 and 9. Histograms showing the numbers of myelinated axons of different diameters in the branches of the dorsal abdominal vagal trunk. The positions at which individual counts were made are shown above.

Two sheep were used, and short lengths of the various nerves were fixed and stained with osmic acid. Counts at each position were made in duplicate, and discarded if any value from corresponding nerves had a discrepancy of more than 5%. Figures given here are mean values.


11000
9000
7000
5000
3000
1000
0
A
1000
0
F
Nos. of fibres
1
2
3
4
5
6
7
8
Ext. diam. (μ)


2000
1000
0
B
3000
2000
1000
0
C
2000
1000
0
D
2000
1000
0
E
Nos. of fibres
1
2
3
4
5
6
Ext. diam. (μ)

Ventral vagus

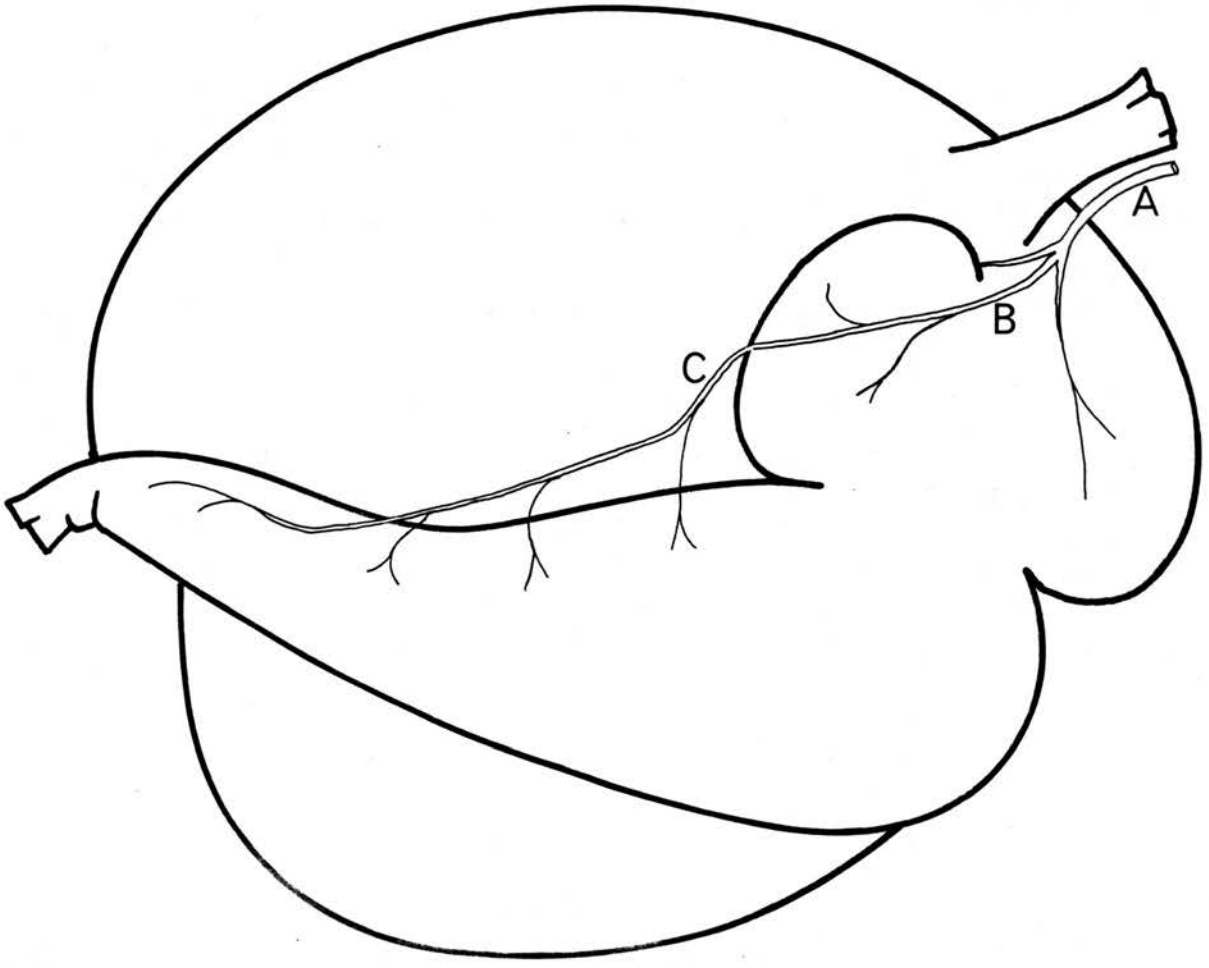
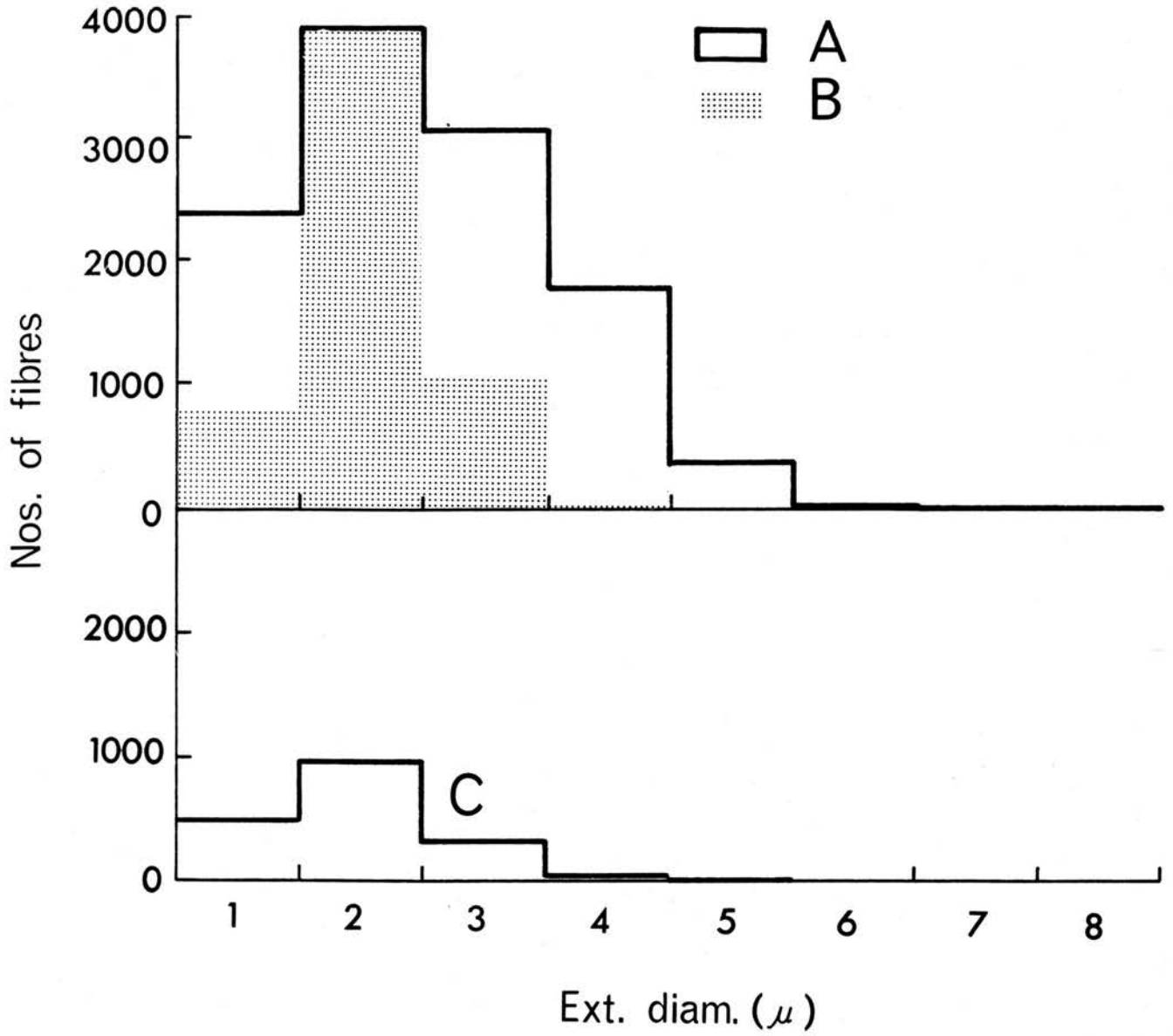


Fig. 10. Histograms showing the numbers of myelinated axons of different diameters in the branches of the ventral abdominal vagal trunk. The positions at which individual counts were made are shown above. By superimposing the histogram for the nerve supplying the omasum and abomasum on the ventral vagus itself, it is possible to deduce the histogram for the supply to the reticulum.

Only one sheep was used, and short lengths of the various nerves were fixed and stained with osmic acid. Counts at each position were made in duplicate, and discarded if any value from corresponding nerves had a discrepancy of more than 5%. Figures given here are mean values.


4000
3000
2000
1000
0
2000
1000
0
A
B
C
Nos. of fibres
1
2
3
4
5
6
7
8
Ext. diam. (μ)

reticulo-omasal orifice have a higher proportion of 2μ diameter fibres. Myelinated axons running in the right and left ruminal nerves tend to be of smaller diameter (1 to 2μ). If the frequency histogram for myelinated nerve fibres supplying the reticulum is estimated by subtraction, it is found that the histogram lies further to the right than it does for other branches described, and corresponds more to that demonstrated for the dorsal ruminal nerves which have mean fibre diameters of 2 to 3u. Any estimate derived by subtraction, however, ignores the presence of the fine strands marked 3 in Fig. 5. Counts of these have not been made.

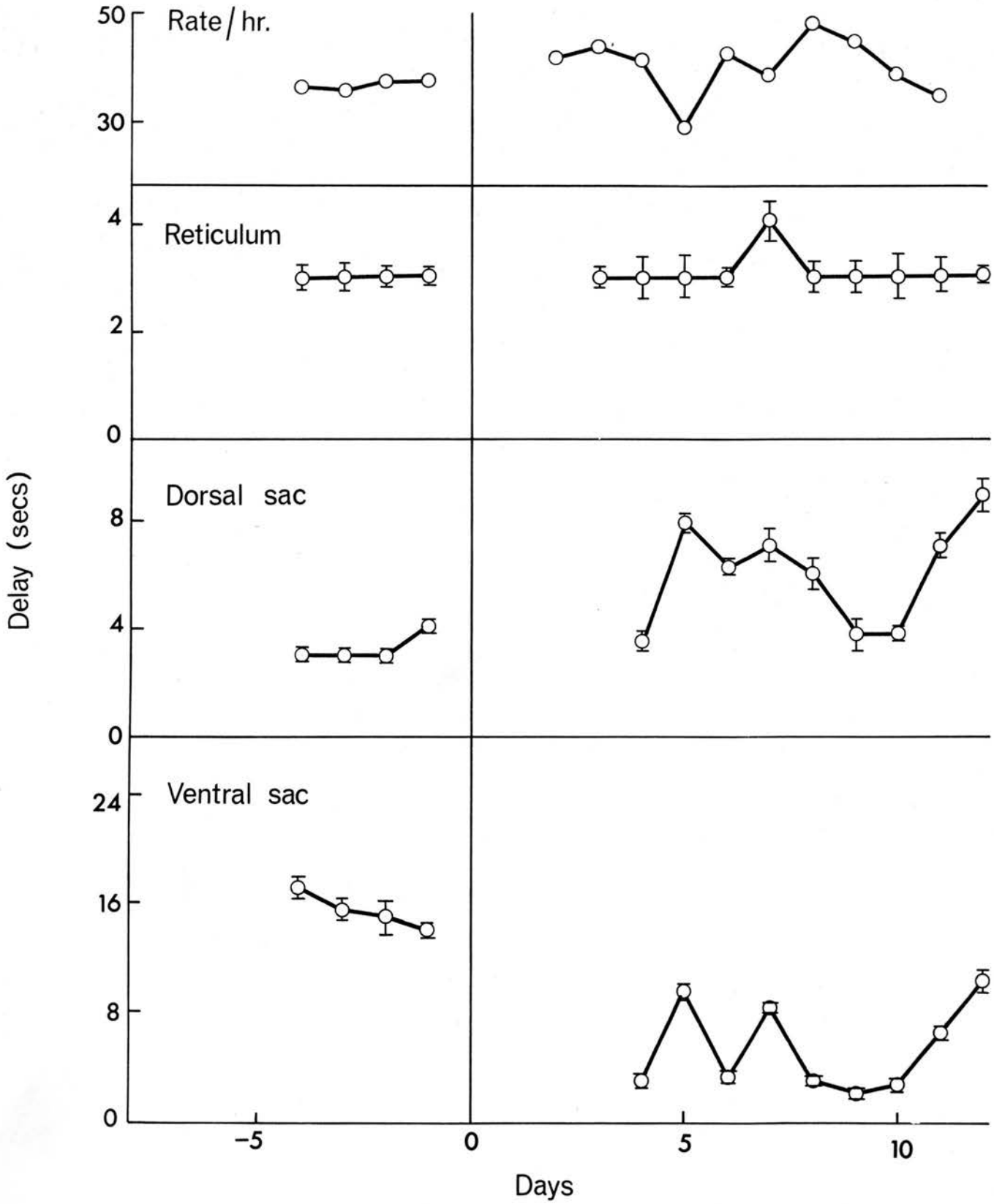
The abomasum receives a large total number of myelinted nerve fibres although as mentioned above, these are mostly of small diameter. The reticulum, however, is profusely supplied with large diameter myelinated axons - the total number of such fibres counted in the various nerves supplying the rumen (14,000) was about the same as the number (13,000) estimated to supply the reticulum.

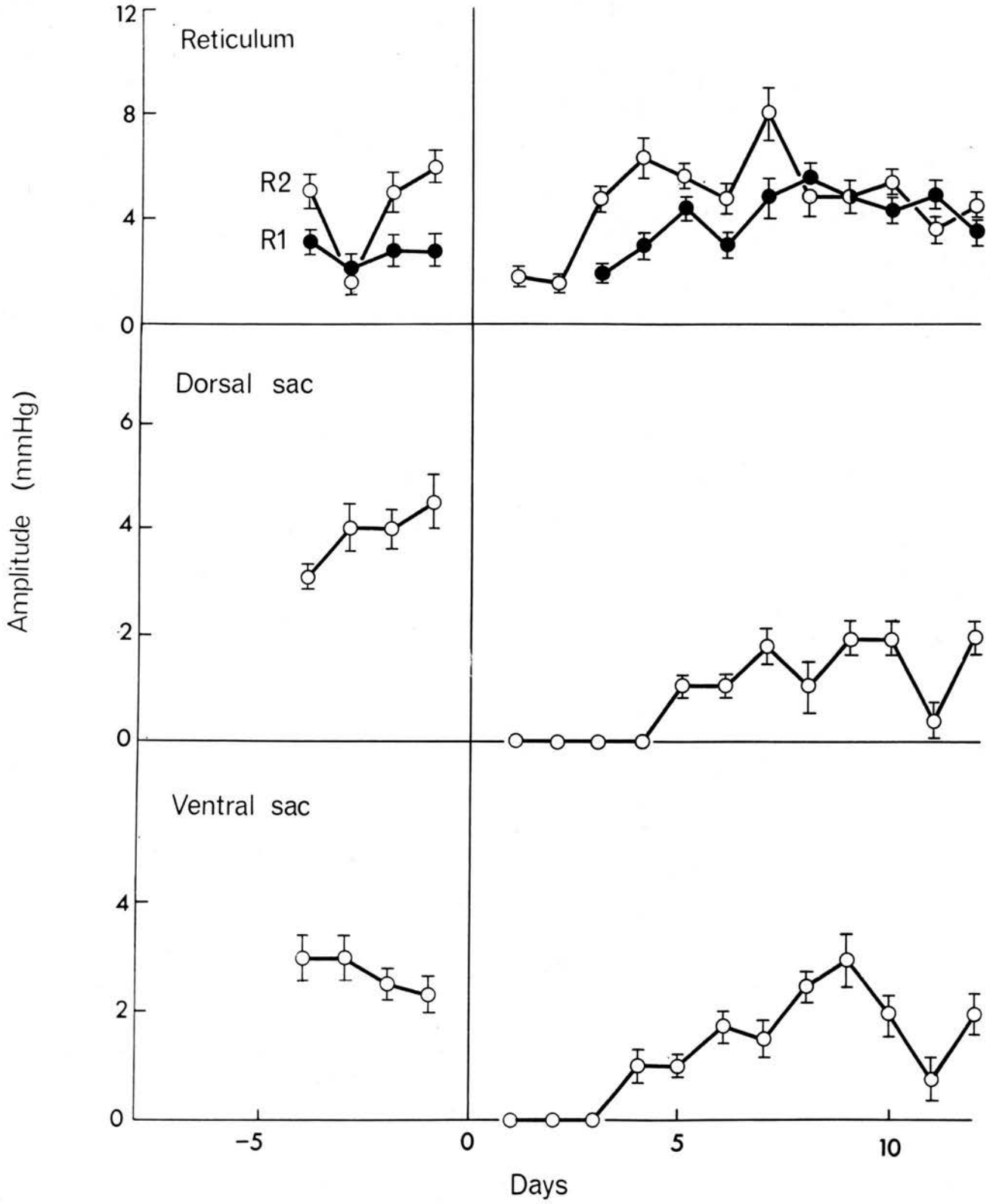
Effects of Section of the Dorsal or Ventral Vagal Trunk on Rhythmic Gastric Motility.

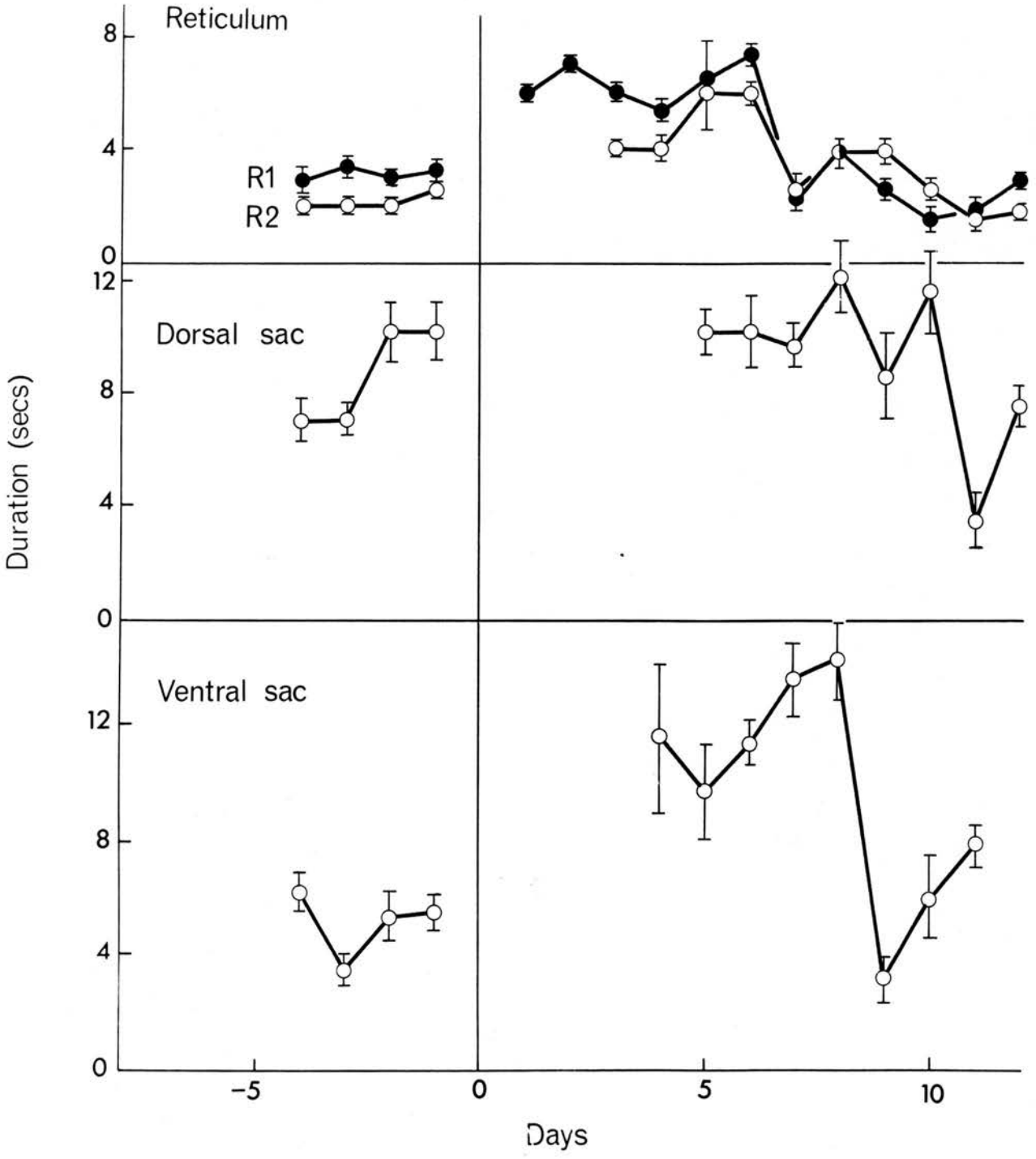
Figs. 11 to 20 show the effects on gastric motility of transection of the vagal trunks. Data have been plotted on a daily basis to show the frequency, amplitude, duration and time relationships of the various components of the contraction cycles during the experimental period. These parameters vary from cycle to cycle, even in control animals. To some extent, this was reduced by taking an average of 10 successive readings from a 'steady' part of each day's recordings; standard deviations refer to variations over this period. The values presented were obtained from 5 sheep in which the dorsal vagal trunk was ruptured, 4 in which the ventral trunk were severed; 2 control animals were subjected to thoracotomy alone. After the first 72 hrs, during which time the food supplied was limited in quantity, daily food intake appeared to be normal.

Figs. 11, 12 and 13. The effects of transection of the dorsal vagal trunk, at the level of the diaphragm, on 'resting' motility of the forestomach. Data presented are mean values from 4 sheep; mean standard deviations for each measurement are indicated by vertical bars. Values are presented for the frequency, delay (from onset of the first pressure rise in the reticulum to the onset of the pressure rise in the compartment indicated), amplitude and duration of each phase in the primary contraction cycle.

In the case of reticular motility, filled circles (R1) represent the first phase of contraction, clear circles (R2) the second phase. Day 0 is the day on which neurectomy was performed.

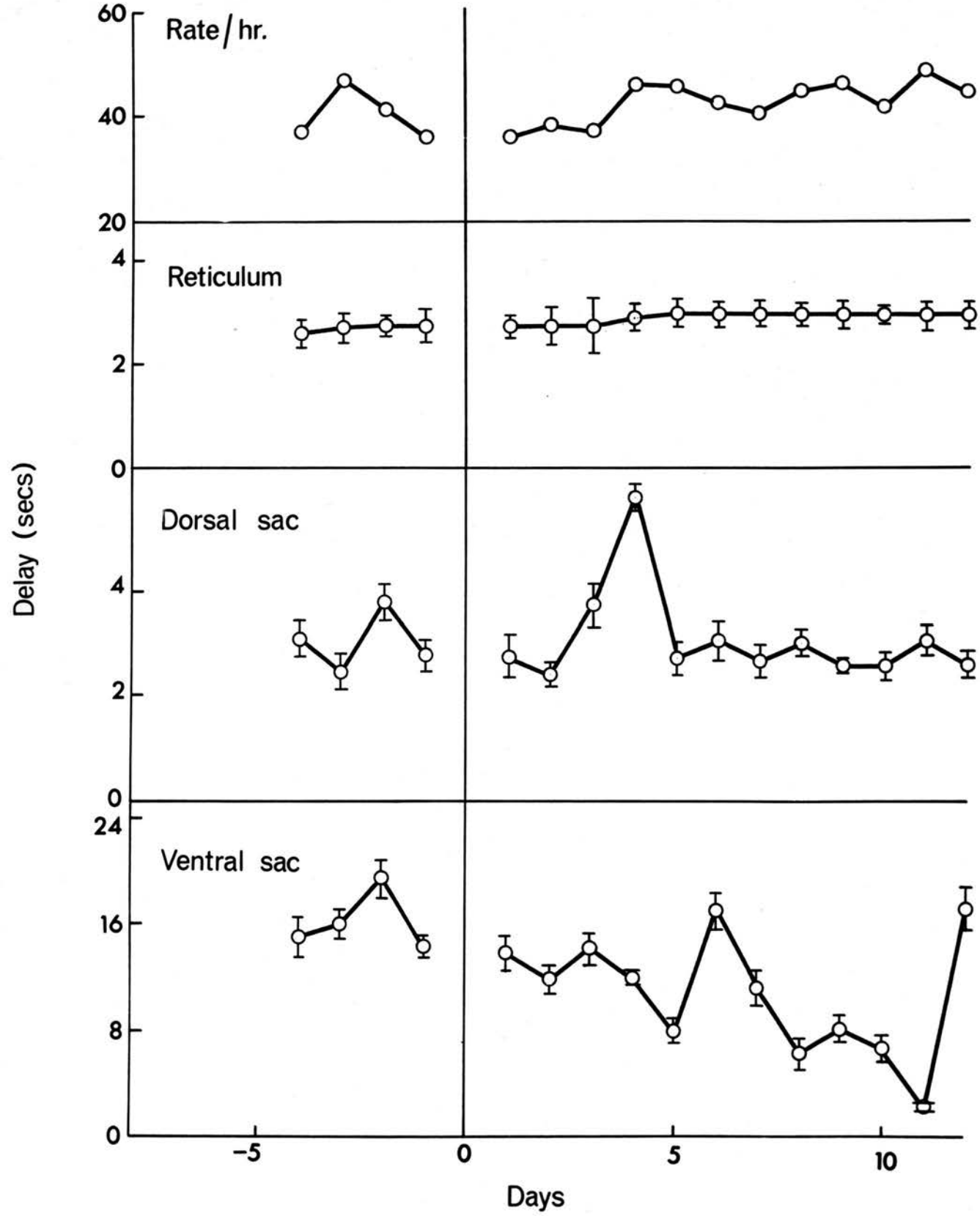

Rate / hr.
50
30
Reticulum
4
2
0
Dorsal sac
8
4
0
Ventral sac
24
16
8
0
Delay (secs)
−5
0
5
10
Days

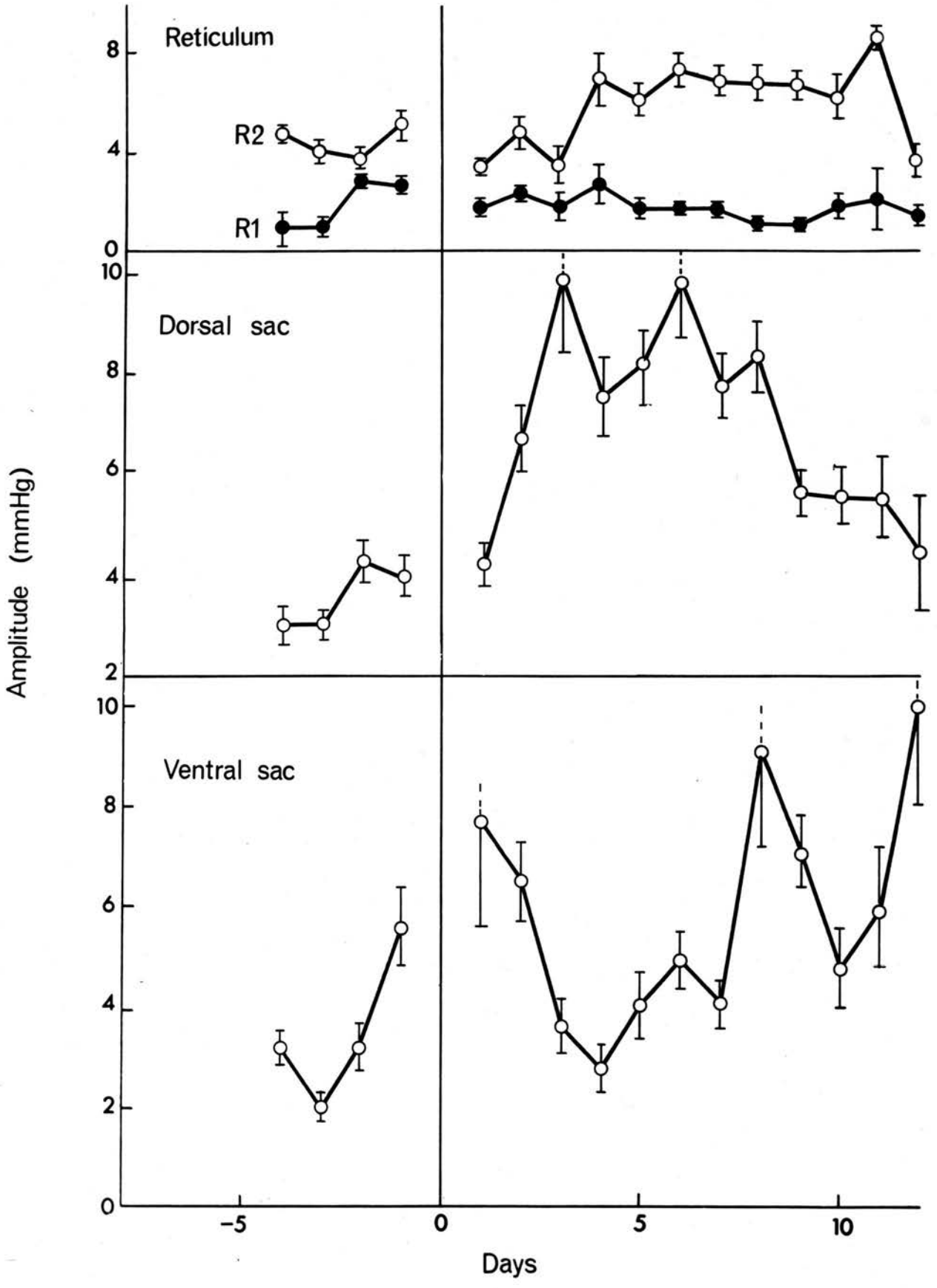

Reticulum
R2
R1
Dorsal sac
Ventral sac
Amplitude (mmHg)
Days
12
8
4
0
6
4
2
0
4
2
0
-5
0
5
10


Reticulum
R1
R2
Dorsal sac
Ventral sac
Duration (secs)
Days
0
4
8
12
-5
0
5
10

Figs. 14, 15 and 16. The effects of transection of the ventral vagal trunk, at the level of the diaphragm, on 'resting' motility of the forestomach. Data presented are mean values from 4 sheep; mean standard deviations for each measurement are indicated by vertical bars. Values are presented for the frequency, delay (from onset of the first pressure rise in the reticulum to the onset of the pressure rise in the compartment indicated), amplitude and duration of the primary contraction cycle.

In the case of reticular motility, filled circles (R1) represent the first phase of contraction, clear circles (R2) the second phase. Day 0 is the day on which neurectomy was performed.


Rate / hr.
60
40
20
Reticulum
4
2
0
Dorsal sac
4
2
0
Ventral sac
24
16
8
0
Delay (secs)
-5
0
5
10
Days


Reticulum
R2
R1
Dorsal sac
Ventral sac
Amplitude (mmHg)
Days
0
2
4
6
8
10
-5
0
5
10

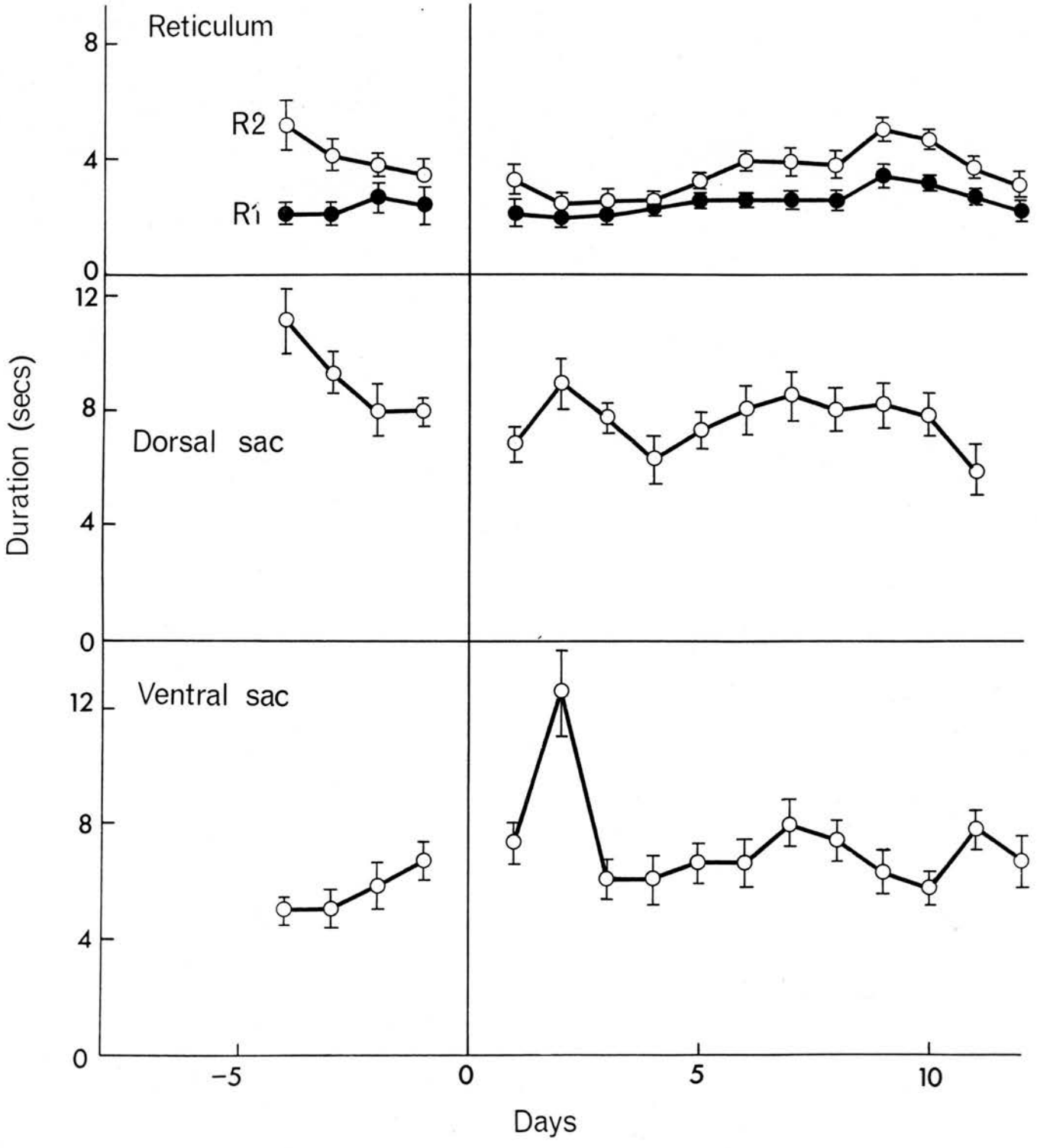

Reticulum
R2
R1
Dorsal sac
Ventral sac
Duration (secs)
Days
0
4
8
12
-5
0
5
10

Fig. 17. Motility of the forestomach of a 1 yr. old Blackface sheep on the day following section of the ventral vagal trunk at the level of the diaphragm. This pattern of motility closely resembles that recorded from control animals, except that the contraction of the ventral sac of the rumen occurs much earlier here than normally. The left half of the trace shows motility during rumination (indicated by regular movements of the jaw), the right half shows 'resting' motility.

THIS RECORDING WAS MADE ON SMOKED KYMOGRAPH PAPER, AND HAS BEEN REVERSED PHOTOGRAPHICALLY. ALL SUBSEQUENT KYMOGRAPH TRACINGS HAVE BEEN TREATED IN THIS WAY.

SHEEP A14 20:2:64

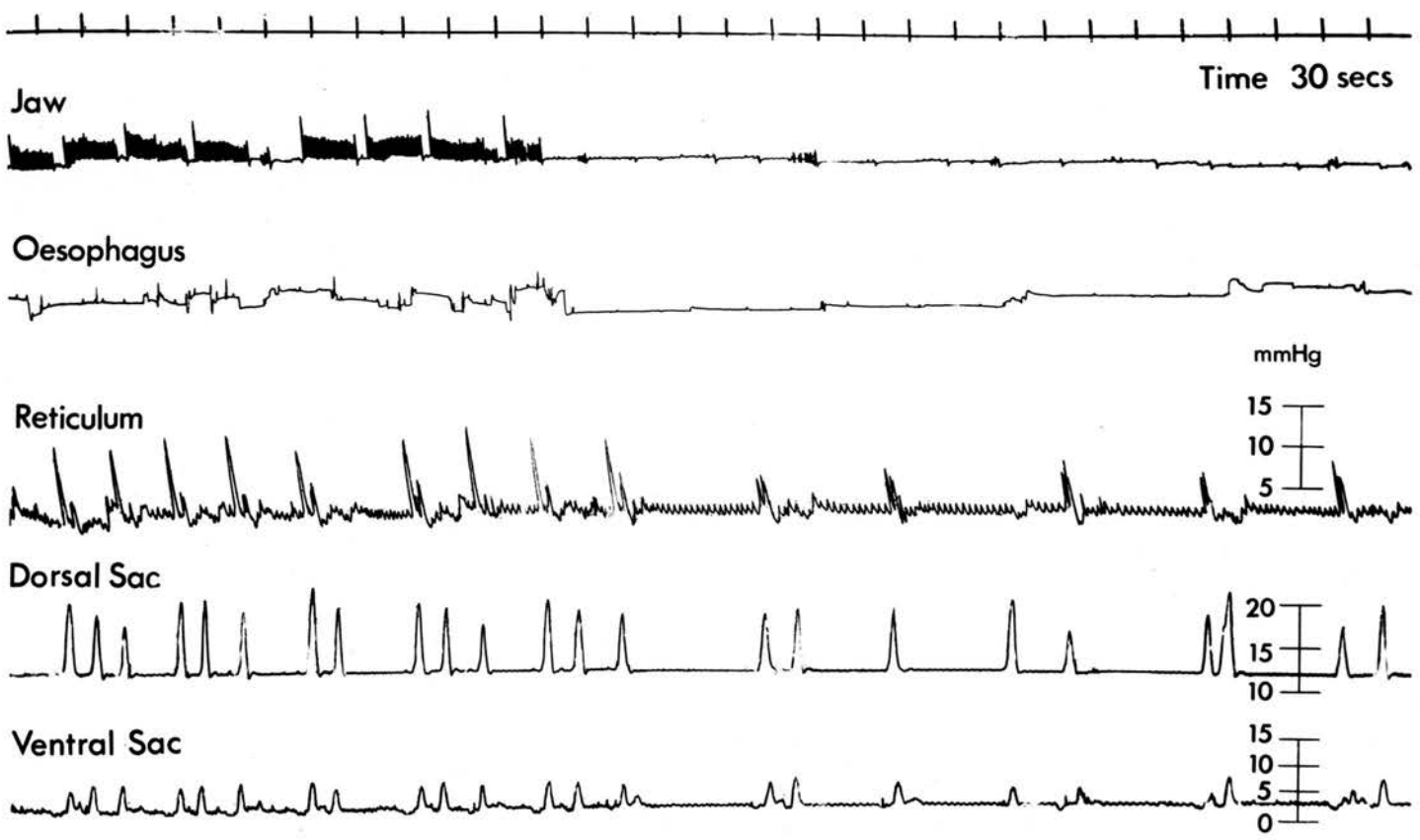


Fig. 18. Kymograph recording showing the contractile events in the forestomach of a 9 m. old Blackface sheep 3 days after section of the dorsal vagal trunk at the level of the diaphrahm. Biphasic contractions of the reticulum are present, but there is no motility of the rumen.

Sheep not ruminating.

SHEEP A5 11:10:63

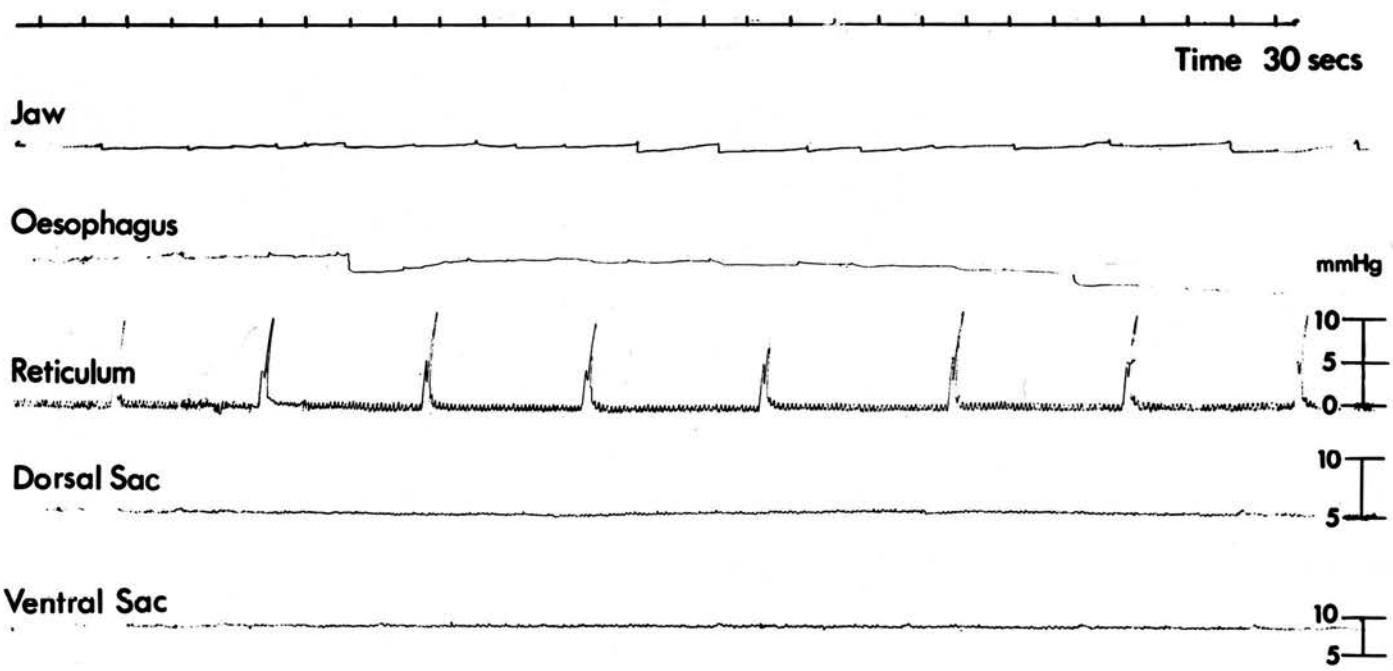


Fig. 19. Kymograph recording showing the contractile events in the forestomach of a 9 m. old Blackface sheep 5 days after transection of the dorsal vagal trunk at the level of the diaphragm.

Biphasic contractions of the reticulum are present, and motility is reappearing in the mid-dorsal and mid-ventral sacs of the rumen.

Sheep not ruminating.

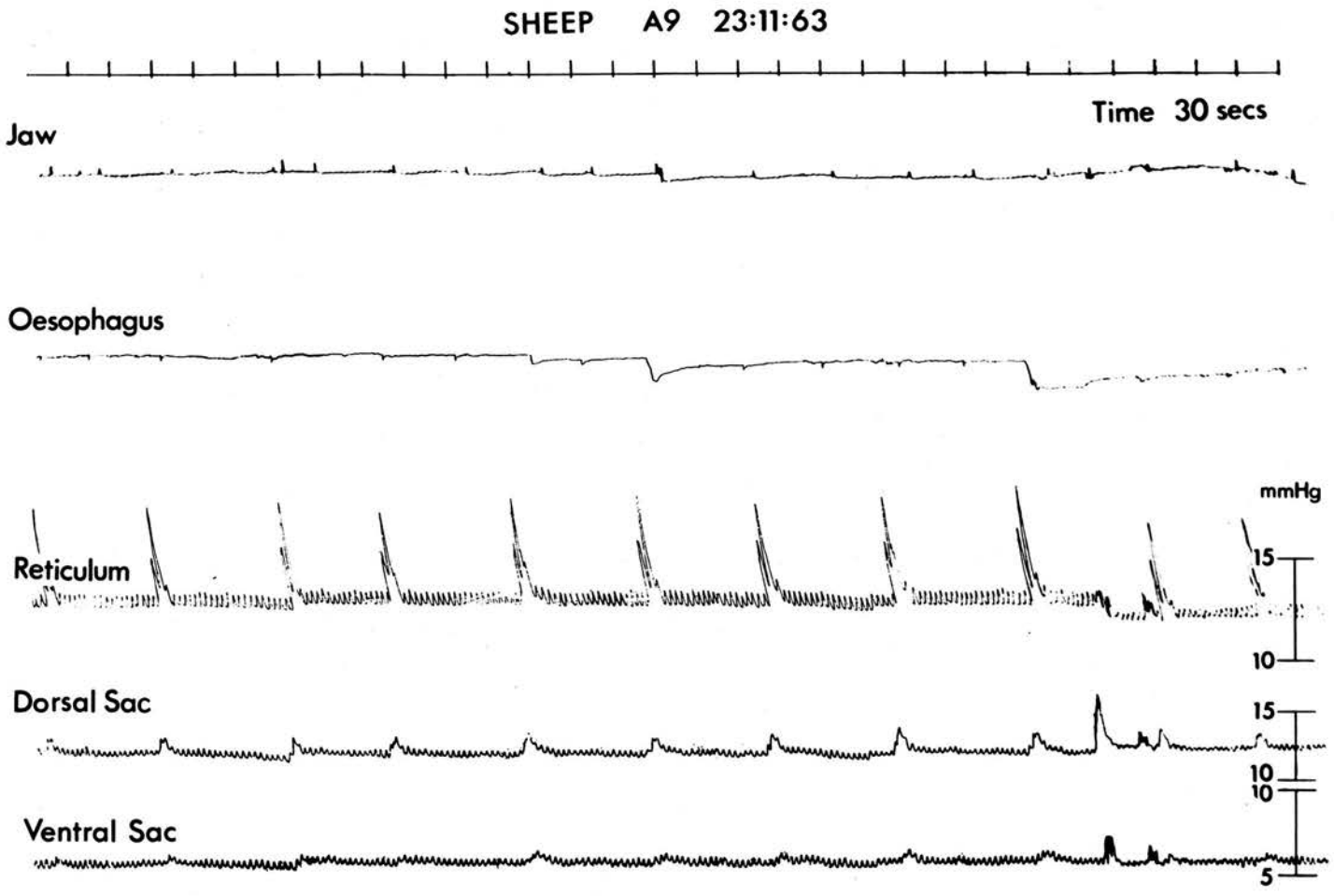

SHEEP A9 23:11:63
Time 30 secs
Jaw
Oesophagus
mmHg
Reticulum
15
10
Dorsal Sac
15
10
10
Ventral Sac
5

Fig. 20. Kymograph tracing from the same sheep as in Fig. 19, 5 days after transection of the dorsal vagal trunk at the level of the diaphragm. Sheep is ruminating. Periods of remastication are regular, and are repeated about once each minute. In this case, contraction of the reticulum appears to be triphasic; this is often recorded from normal sheep. Contractions of the rumen are of very low amplitude.

SHEEP A9 23:11:63

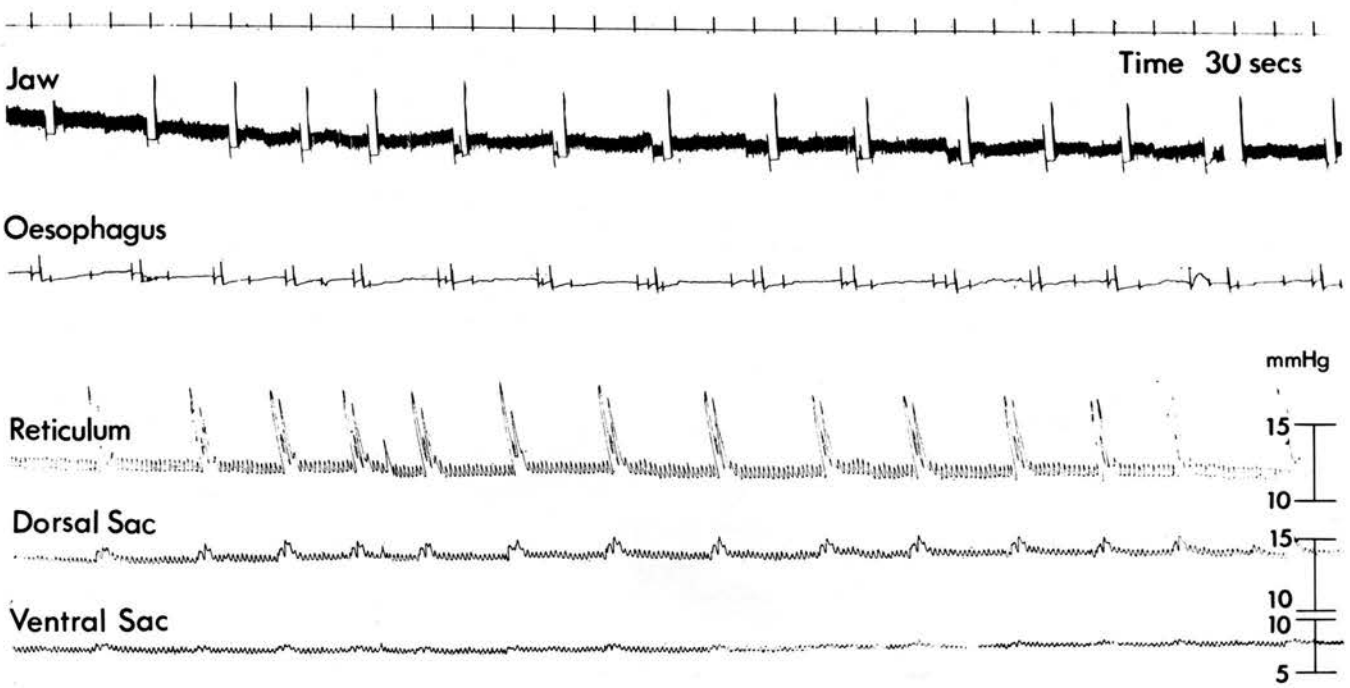
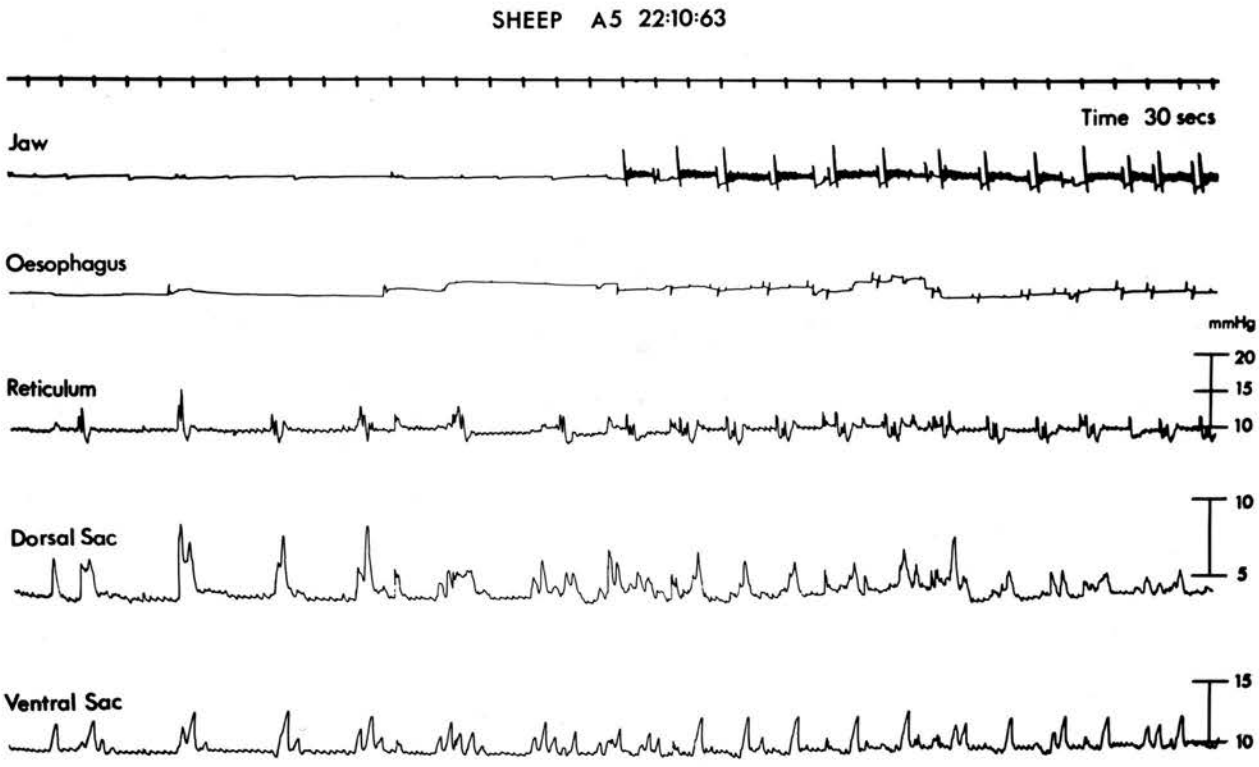


Fig. 20a. Kymograph tracing from the same sheep as Fig. 18, 14 days after transection of the dorsal vagal trunk at the level of the diaphragm. The right half of the trace shows motility during rumination (indicated by regular movements of the jaw), the left half shows 'resting' motility. This pattern of motility is similar to that recorded from control animals.


SHEEP A5 22:10:63
Time 30 secs
Jaw
Oesophagus
mmHg
20
15
10
Reticulum
10
5
Dorsal Sac
15
10
Ventral Sac

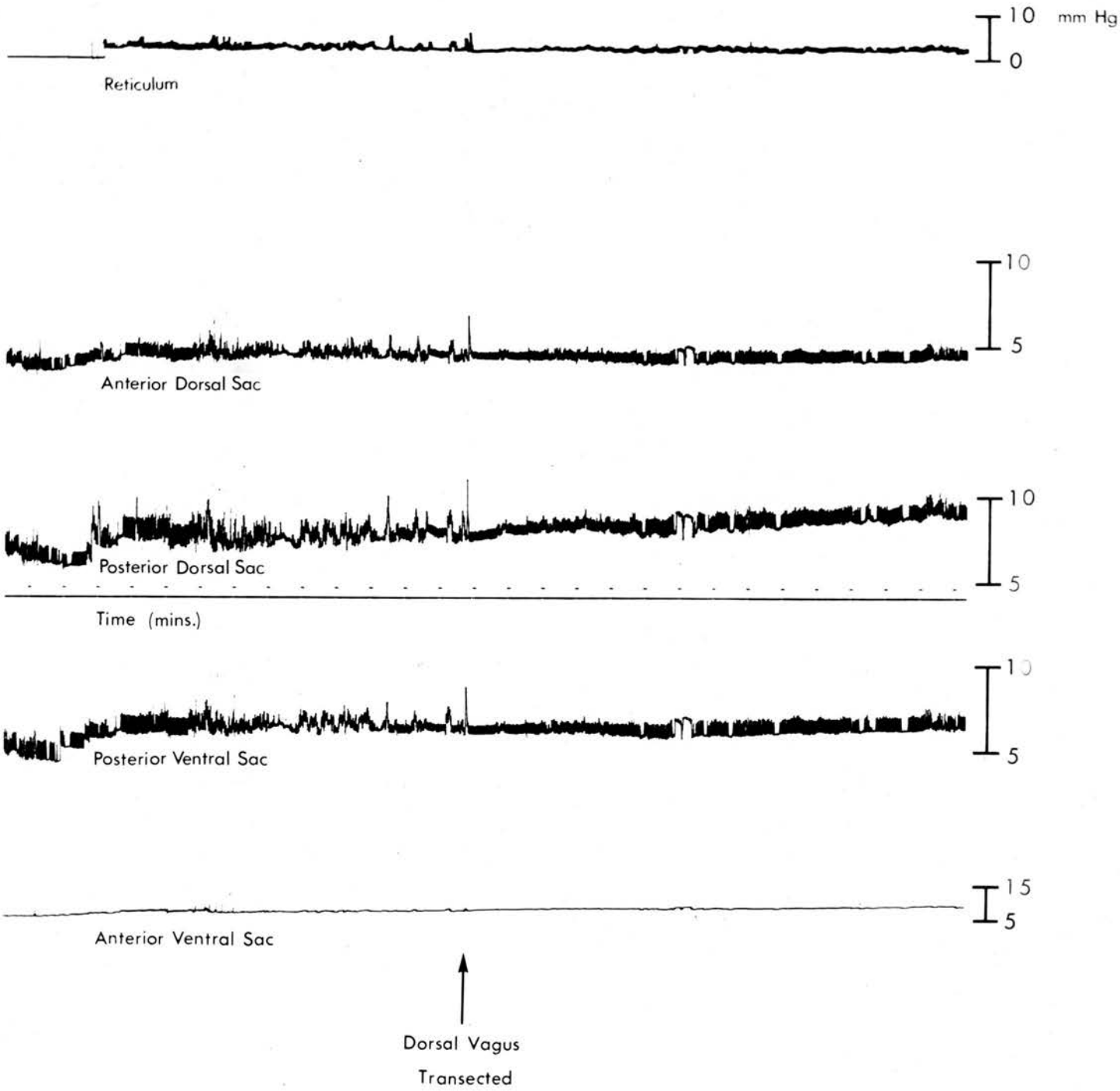
In the control animals, thoracotomy did not significantly alter the frequency, amplitude, duration or time relationships of the different components of the forestomach contraction cycle. Between the 3rd and 7th days the interval between contraction of the reticulum and contraction of the ventral sac of the rumen varied between 3 and 21 sec. (control level 26 sec.), but statistically this was not significant (P 1/ 100). It is therefore justifiable to use data obtained from each animal before neurotomy as a control for observations following neurotomy.

Interruption of the ventral vagal trunk had no significant effect on any of the measured parameters of the contraction cycle (P. 1/ 100), although in three animals there was an increase in the amplitude of contraction of the dorsal sac of the rumen over a period of 10 days.

One sheep was anaesthetised with Halothane, with the recording balloons in their respective positions in the forestomach. Rhythmic gastric contractions disappeared during anaesthesia, but were re-established by distension of the balloon in the reticulum with 400 ml. air. Dorsal vagotomy caused an immediate disappearance of motility from the rumino-reticulum (see Fig. 21). In this, and two other animals, in which the dorsal vagus had been transected, gastric contractions disappeared completely for 2, 2 and 4 days respectively. In the other two, rumen motility was abolished although the reticulum periodically underwent monophasic contractions lasting 6 to 9 sec. for 3 days after vagal resection; the frequency of these contractions was identical with that of primary cycles before neurotomy. After the number of days indicated above, biphasic contractions of the reticulum reappeared, and 1 to 2 days after this, the rumen began to contract, pressure within its lumen rising 1 to 2 mm. Hg.,

Fig. 21. Pen-recorder tracing showing the effect of transection of the dorsal vagal trunk (arrow) on motility of the rumino-reticulum. Balloons were placed in (from above downward) reticulum, anterior and posterior dorsal sacs of the rumen, and posterior and anterior ventral sacs of the rumen.

The sheep (1 yr. old Blackface) was anaesthetised with Halothane, and contractions of the forestomach were induced by inflating the balloon in the reticulum with 400 ml. air. Note the abolition of motility at all 5 points.


10
mm Hg
0
Reticulum
10
5
Anterior Dorsal Sac
10
5
Posterior Dorsal Sac
Time (mins.)
10
5
Posterior Ventral Sac
15
5
Anterior Ventral Sac
Dorsal Vagus
Transected

this increasing to 3 mm. Hg. in the ventral sac of the rumen by the 9th day. The duration of the contractile event in the reticulum and dorsal sac of the rumen slightly increased for about 4 days after the reappearance of motility in the respective compartments; this was not statistically significant. Subsequently, these contractions had a similar duration to that during the control period. The interval between the onsets of contractions of the reticulum and dorsal rumen sac was not significantly altered (approximately 5 sec.). When contractions were re-established in the ventral sac of the rumen, they occured within 8 sec. of the reticulum contraction (14 sec. before vagal transection) and the duration of contractions rose from 5 to about 12 sec. until the 9th day, when it fell abruptly to the control level. Both of these findings were statistically significant ($P < 1/100$).

DISCUSSION

The anatomical observations confirm the description given by Habel (1956) of the innervation by the vagus nerve of the ruminant stomach, except that no interchange of nerve fibres between the two trunks was found within the abdomen. As the smallest nerves traced measured 0.2 x 0.4 mm., it is possible that fine branches passed to the rumen from the ventral vagal trunk, even though no such fibres were found. Electrical stimulation of this vagal trunk failed to cause contraction of the rumen so that if this trunk does supply motor fibres to the rumen, they are not very abundant.

Transverse sections of vagal branches supplying the four gastric compartments, reveal that each compartment tends to be supplied by myelinated nerve fibres of a particular size spectrum - myelinated fibres running to the rumen and abomasum are mostly of small diameter (1 to 2μ) although the cardia and dorsal rumen sac receive a large number (7,000 to 8,000) of larger (2 to 3μ) myelinated axons from the dorsal vagal trunk. The pre-diaphragmatic anastomosis between dorsal and ventral trunks of the vagus contains 1,500 to 2,000 large diameter axons which are so closely packed that there cannot be an extensive exchange of amyelinate fibres. It is unlikely that many very large axons would pass to the rumen. If additional fibre interchange were to occur between the two vagal trunks as suggested by Habel (1956) it would be expected to involve axons of a similar size spectrum. A similar argument could be applied to them.

The alterations in motility of the rumen following dorsal vagotomy are dramatic. All contractions disappear for 3 to 4 days, and after this period, reappear with a low amplitude. In about 5 days the ventral sac of the rumen may contract, as

strongly as before neurotomy, but contractions begin much earlier, relative to the first contraction of the reticulum, and the duration of each contraction is approximately doubled. Although rumen contractions remain of relatively low amplitude, their durations and times of onset are not significantly altered.

If recovery were to be a result of hypersensitivity, motility would be expected to reappear in 3 to 4 days (Cannon & Rosenblueth, 1949). The amplitude of contraction under such circumstances would depend on the extent of the intact innervation but each contraction would be expected to last longer than in control animals. After the reappearance of motility in the case of reticulum, dorsal and ventral sacs of the rumen, each contraction did last longer, but by the 10th post-operative day all the values were normal. This could not be predicted on the basis of a general hypersensitivity. The reduction of the interval between contraction of the reticulum and ventral sac of the rumen would not be expected if the return of motility was due to degeneration hypersensitivity, nor if it was due to a normally functional 'peripheral pacemaker'.

In conclusion, there is no direct evidence that the ventral vagal trunk innervates the rumen. The return of motility of the rumen following transection of the dorsal vagal trunk has certain features in common with the development of hypersensitivity, associated with a persistent nerve supply, but on about the 10th day following nerve resection these factors disappear. If the resumption of activity by a 'peripheral pacemaker' is involved, it is difficult to explain the altered relationships of the contraction cycle; it should be realised however, that contraction cycles with these time relationships have been observed in normal ruminants (see e.g. Seren, 1959), and they need not, therefore, indicate a fundamental modification of the discharge of the reticulo-ruminal centre.

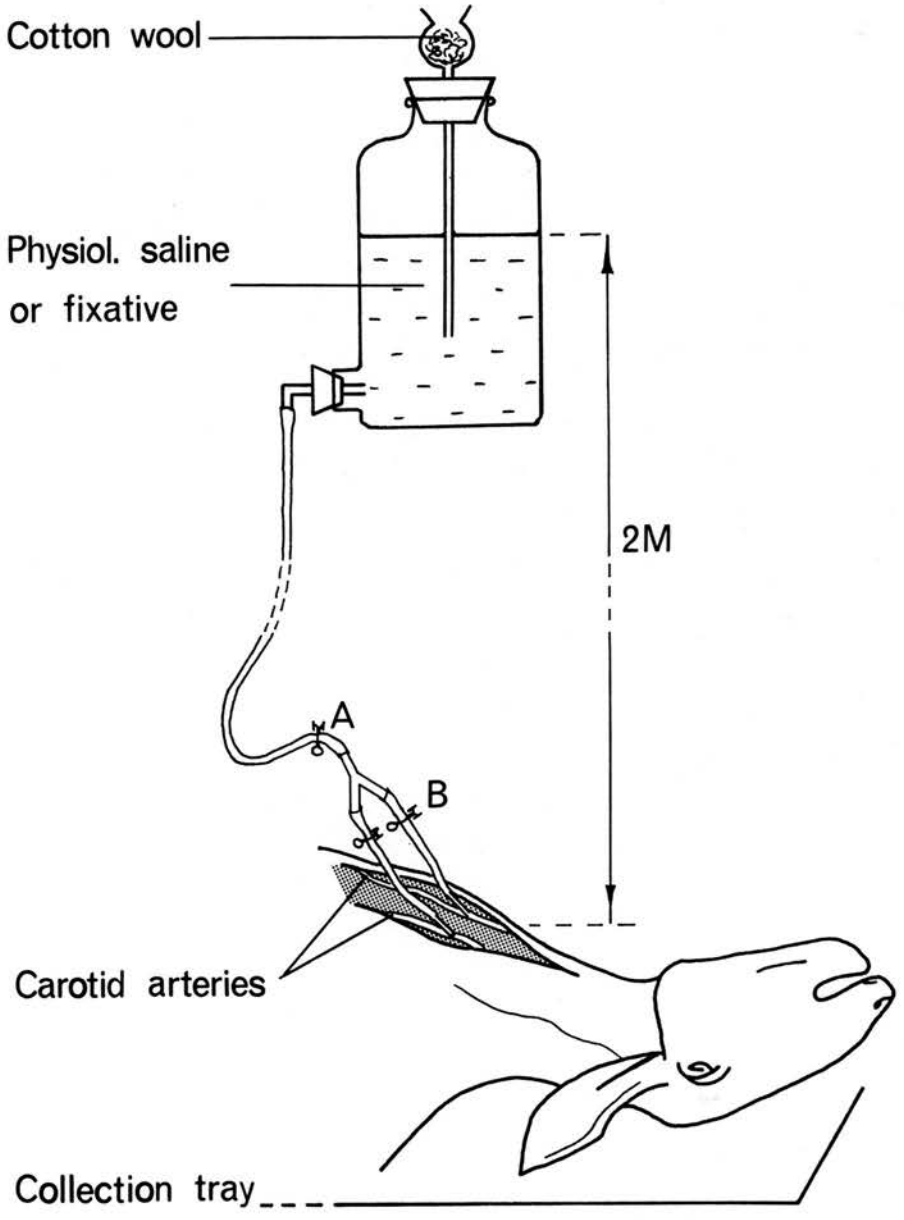
SECTION 3

Sheep used in the experiments to be described in this section were maintained in the manner described in Section 2. Material for the determination of the positions of the cells of origin of the vagal trunks was obtained for the same animals as those used to demonstrate the effects on motility of the rumino-reticulum, of section of these nerve trunks. Details of these animals are presented in Section 2.

Perfusion and Fixation of the Head.

On the 14th post-vagotomy day, when Nissl lysis is most pronounced, (see e.g. Bell, 1960; Szabo & Dussardier, 1964), experimental animals were deeply anaesthetised using pentobarbitone sodium, brand Sagatal (50 mg./K i.v.). The animal was then placed in dorsal recumbency, and a midline ventral incision made longitudinally in the neck. Two threads were passed around each common carotid artery and the one nearer the heart was tightened to occlude blood flow. The perfusion apparatus shown in Fig. 22 was completely filled with physiological saline and clip A was closed, while both clips B remained open; the glass cannulae were then inserted anterior to these ligatures, and tied in position so that the whole head could be perfused with 0.9% saline. The perfusion bottle was raised to a height of 2 metres, and the clip (A) opened to allow saline to enter both arteries simultaneously; both jugular veins were cut in the mid-cervical region, and 2 L of saline were allowed to enter the carotid bed - perfusion time varied from $1\frac{1}{2}$ to 2 min.; at the end of this time the fluid leaving the jugular veins was almost clear. Clip A was now closed, the perfusion bottle was refilled with $2\frac{1}{2}$ L of 10% formol saline, to which was added 1% acetic acid (this fixative does not keep well, and was freshly prepared

Fig. 22. The arrangement used to perfuse the head with fixative in preparation for histological studies.


Cotton wool
Physiol. saline
or fixative
2M
A
B
Carotid arteries
Collection tray

on each occasion). Two and a half litres of formol saline were then allowed to perfuse through the system, and the animal was then decapitated, and the brain, with both vagus nerves attached, was dissected out.

In two experiments to determine the efficiency of this perfusion process, 0.5% toluidin blue was added to the formol saline. Subsequent gross examination of the brain revealed marked staining of all areas anterior to the medulla oblongata; the intensity of staining fell progressively posterior to the anterior margin of the medulla, and was not apparent more than 4 cm. behind the obex. Increasing the height of the perfusion bottle to 3 metres in one additional experiment did not alter the distribution of stain.

The rootlets of the vagal trunks were transected, and each nerve was labelled; the first 5 cm. of each was placed separately in acid formol saline. The cerebrum and cerebellum were removed, and the brain stem was divided transversely into four portions - medulla oblongata, pons, mid-brain and the remainder of the brain stem anterior to this. The medulla was then pinned to a cork board, and orientated to lie in the longitudinal plane of a Prior micro-manipulator; a pin was inserted into the holder of this, and the point coated with Indian ink. A series of 4 mm deep stabs was prepared longitudinally along the course of the dorsal median sulcus, at 2 mm intervals, and was subsequently located on the histological sections to indicate the degree of distortion during processing. The different parts of the brain stem were then placed separately in acid formol saline.

Histological Techniques to Demonstrate Chromatolysis

A number of stains are available to demonstrate Nissl granules in neurons, the most frequently used of which are cresyl violet and toluidin blue. The technique adopted for these

investigations was staining by toluidin blue, as colouration is more easily controlled, and hence results are more consistent than with cresyl violet. Standard histological text-books list various ways of staning with toluidin blue; in a preliminary trial it was found that none of these gave sufficiently consistent results for use in the present experiments. Several modifi-:cations of the techniques were then tested, varying each stage independently, and the most satisfactory procedure was selected.

Material was permitted to remain in fixative for 3 days, and was then washed thoroughly in 1% saline, and then in distilled water. Dehydration was achieved by gradual transfer to progressively stronger alcohol, and was protracted over 4 days. The tissue was 'cleared' in benzene, and embedded in paraffin wax (M.P. 49°C); serial transverse sections were prepared at 20µ on a Reichert rotary microtome model OmS. Sections were attached in series of five to microscope slides using Meyer's egg albumin, such that each slide represented 100µ.

The technique finally adopted for staining the Nissl material was as follows:-

1. Remove wax with two changes of xylol (25 sec.each).
2. Hydrate with descending strengths of alcohol and wash in distilled water.
3. Stain for 30 min. in 1% toluidin blue, maintained at 54°C in a thermostatically controlled oven.
4. Wash with two changes of distilled water.
5. Transfer straight to 95% alcohol for 20 sec.
6. Differentiate in absolute alcohol until sections are just colourless to the naked eye (about 2 to 3 min.).
7. Rinse briefly in fresh absolute alcohol, clear xylol and mount with D.P.X. (Gurr).

The above procedure gave consistently good results, but it is important that the toluidin blue is freshly prepared (it should not be kept for more than 2 weeks), and that the stain is of the correct grade - most satisfactory staining was obtained using the preparation - Chroma-Gesellschaft, Schmidt & Co.

Examination of these slides was performed under the low power (2.5 cm) objective of a standard Zeiss G.F.L. microscope, with a x 6 eyepiece and using a Beck Camera Lucida. The procedure was to sketch the outline of the section on paper, using the Camera Lucida, and then to mark the position of each cell on this; cells considered to be showing chromatolysis (i.e. lysis of the Nissl substance, rounding of the contour, and margination of the nucleolus) were marked in red, whereas unaffected cells were indicated in black. Total cell counts, and the number of degenerating cells were indicated alongside each drawing. Each 5th section was examined in this way, to ensure that no cell was counted twice, and histograms were subsequently plotted to show the distribution of degenerating neurons.

Histological Technique for Cyto-architectonics

The Golgi histological method used was a modification of that suggested by Cox (1891), as laid out by Carleton & Drury (1957). Transverse sections of the medulla oblongata of two sheep were cut at 200µ with a Reichert freezing microtome, type OmE, after soaking for two days in a 20% solution of neutral dextrin; sections were counterstained for 5 min. with a 1% aq. solution of acid fuchsin. After gradual dehydration over a period of 2 hr., the sections were individually mounted in cedarwood oil. Two series of sections were made in this way of the ovine meduallа oblongata, between levels 5 mm. posterior and 5 mm. anterior to the obex.

A representative portion of the dorsal vagal nucleus from alternate sections was photographed through a red filter onto R 20 panchromatic plates, using a Zeiss Standard Universal Microscope with a Zeiss 2.5 cm. objective, and with the condenser removed. Plates were developed in "Demancy's" D.V.P. developer at 20°C for 3 min., fixed, washed and dried in the usual manner. Photographic enlargements were prepared from each slide, such that 1 cm. on the photograph represented 100µ on the sections.

A number of well impregnated cells from alternate slides were touched up with Indian ink, and displayed alongside a horizontal section of the medulla oblongata at the appropriate levels.

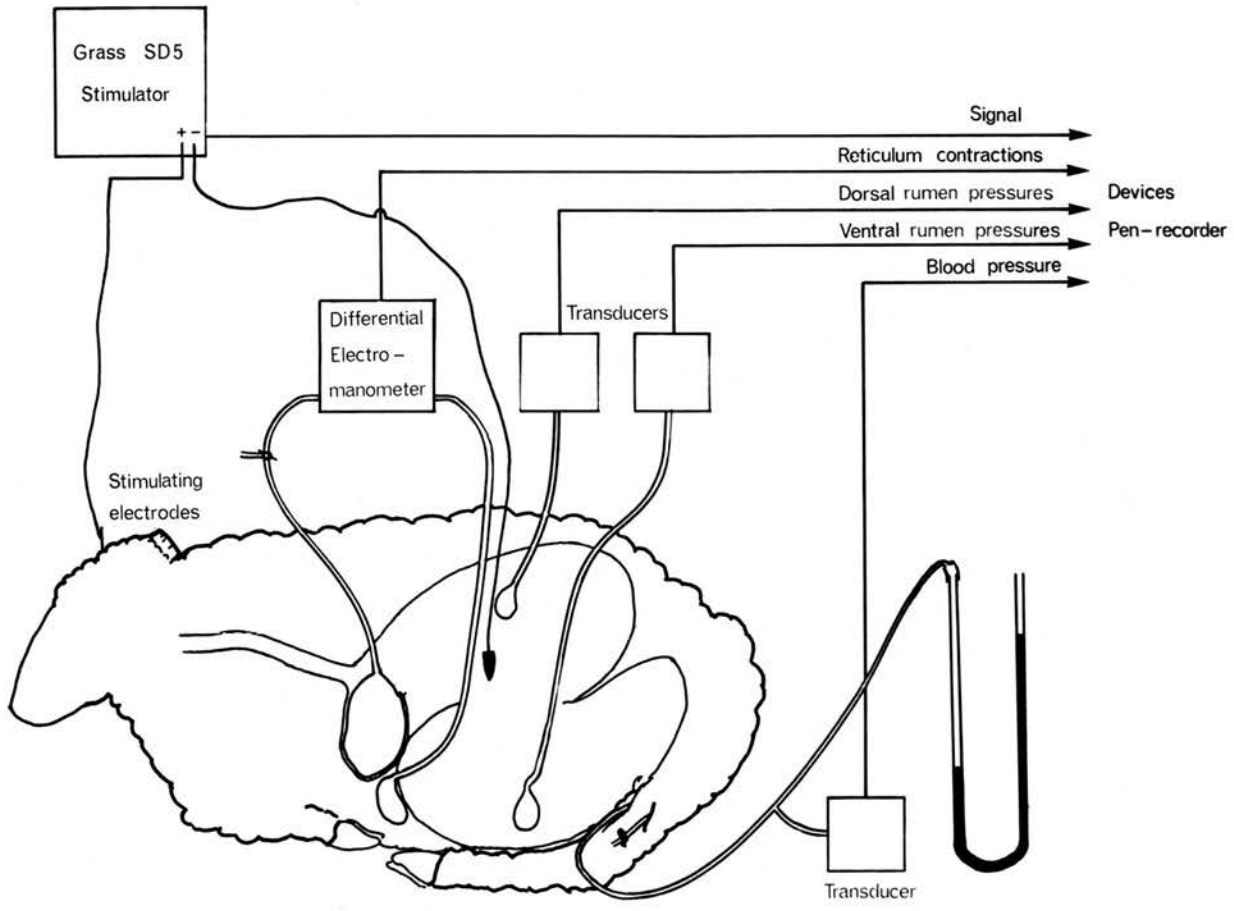
Neurophysiological Preparation

Anaesthesia was induced with Halothane, delivered from a shaped, latex face-mask, but for maintenance a tracheal cannula was inserted, and a "to-and-fro" closed circuit established. A rumen fistula was prepared from the left flank, just below the 3rd lumbar transverse process, and air-filled balloons were introduced into the mid regions of the dorsal and ventral sacs of the rumen these activated C.E.D. strain-gauge transducers, the outputs of which were recorded on an 8-channel Devices pen recorder (see Fig. 23). Pressures within the reticulum were monitored by leading from a water-filled balloon, inserted into the reticulum from an incision in its fundus, to one side of a Greer-type differential manometer, the other side of which was fed by a similar balloon placed immediately adjacent to the reticulum, in the abdominal cavity. The arrangement was such that measured volumes of water at 38°C could be introduced into the balloon inside the reticulum to elicit rhythmical contractions of the forestomach when these were required. In early experiments, pressure changes were recorded on a kymograph using Marey tambours, although this arrangement precluded the recording of differential pressures across the wall of the reticulum.

A cannula was inserted into the fundus of the abomasum, so that measured volumes (usually 100 ml.) of N/10 hydrochloric acid could be added as desired. Blood pressure was continually monitored from the anterior tibial artery, and recorded alongside lead II of the e.c.g. on the pen recorder.

The head of the animal was firmly fixed into a stereotaxic device, with location points at the ears, lower margins of the orbits and the hard palate. Decerebration was performed at a level immediately anterior to the colliculi, which was sufficiently high to avoid decerebrate rigidity and much of the

Fig. 23. Schematic diagram of the experimental arrangement for electrical stimulation of the dorsal vagal nucleus.


Grass SD5
Stimulator
+ −
Signal
Reticulum contractions
Dorsal rumen pressures
Ventral rumen pressures
Blood pressure
Devices
Pen-recorder
Differential
Electro-
manometer
Transducers
Stimulating
electrodes
Transducer

haemorrhage which was encountered with section at more posterior levels, but did not result in limb movements following withdrawal of the anaesthetic. The most satisfactory technique was to occlude both carotid arteries in the mid-cervical region, and following exposure of the dura mater over the cerebellum and posterior cerebral hemispheres, to ligate the longitudinal sagittal sinus. It was now possible to perform decerebration and, by puncturing the cerebellomedullary cistern, to remove the cerebro-spinal fluid which tends to accumulate here. This caused the cerebellum to collapse somewhat, and the overlying dura mater was easily dissected off, and the entire cerebellum removed by suction. Haemorrhage was initially controlled by the application, with pressure, of cotton wool pads, but as the extent of extravasation was reduced, oxidised cellulose gauze ("Surgicel") was applied to the cut faces. With this procedure, blood flow through the carotid arteries could usually be restored within 5 min. The surface of the medulla was now prepared for electrical stimulation under a layer of liquid paraffin.

It was sometimes found that rhythmic contractions of the reticulum could be recorded following such procedures, usually within 10 minutes of withdrawing the anaesthetic. At first, luminal pressure changes associated with these were of low amplitude (about 0.1 to 0.2 mm.Hg.) but they became progressively larger over succeeding contraction cycles until, after a further 10 minutes, rhythmic pressure rises of 5 to 10 mm. Hg. were present, and the characteristic biphasic pattern was usually evident.

When motility of the reticulum was established during preparation of the animal, or within a few minutes of discontinuing the flow of anaesthetic, each contraction was indicated by a single rise in pressure of up to 10 mm. Hg.; this was sometimes followed by one or more smaller pressure

peaks. Decerebrate preparations, however, showed a pattern of motility of the reticulum which resembled that recorded in conscious animals viz: a relatively small rise in pressure of up to 5 mm. Hg., followed by a higher pressure peak of similar duration, up to 15 mm. Hg. in amplitude. As the mean diastolic blood pressure fell toward 50 mm. Hg. the first phase of the complex tended to disappear; the exact level at which this was lost varied considerably in different animals, and it is quite possible that factors other than blood pressure were involved.

In general, it was found necessary to provide some form of afferent stimulation in order to establish motility. Distension of the balloon in the reticulum with warm water was particularly efficacious in this respect, although the volume of water required varied markedly in different animals; volume required usually varied between 200 and 1200 ml. (see Fig. 24). Another procedure which often proved valuable in the elicitation of contractile sequences was acidification of the abomasal contents with hydrochloric acid, or drainage of this gastric compartment using the cannula mentioned earlier (Fig. 25). In order to prevent progressive increase in the volume of the abomasal contents during the course of an experiment, the organ was evacuated before each subsequent addition - usually, if rhythmic contractions had not appeared when the pH (as measured on an E.I.L. Vibrette pH meter) of the contents had fallen to 1.5, further reduction of pH by addition of more acid did not prove to be an adequate stimulus in itself. If rhythmical contractions were already present, further acidification of the abomasal contents tended to increase their frequency, whereas further distension of the reticulum generally increased their amplitude. Gross distension of the reticulum caused a reduction in amplitude and frequency of reticulum contractions; the volume at which this appeared varied greatly in different animals.

Fig. 24. Pen-recorder tracing showing the appearance of rhythmic contractions of the reticulum (upper trace) when the volume of water present in the balloon in its lumen is increased from 400-600 ml. Decerebrate sheep. Contractions of the dorsal sac of the rumen show a much closer temporal relationship with reticular motility than do contractions of the ventral rumen sac. Blood pressure recorded from the anterior tibial artery; a correction of -10 mm. Hg. must be made in this case to offset the difference in levels between the manometer and the tip of the catheter.

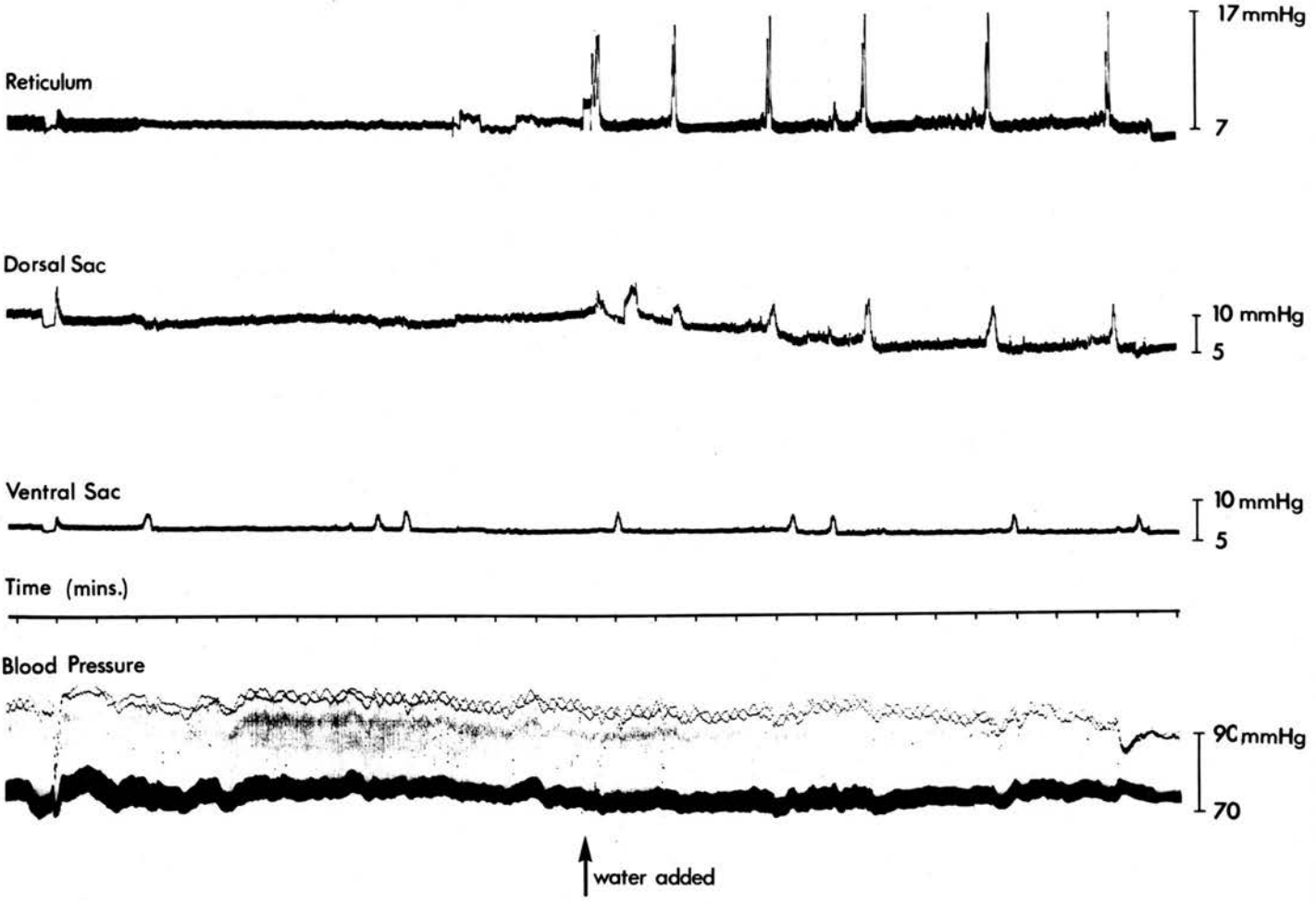
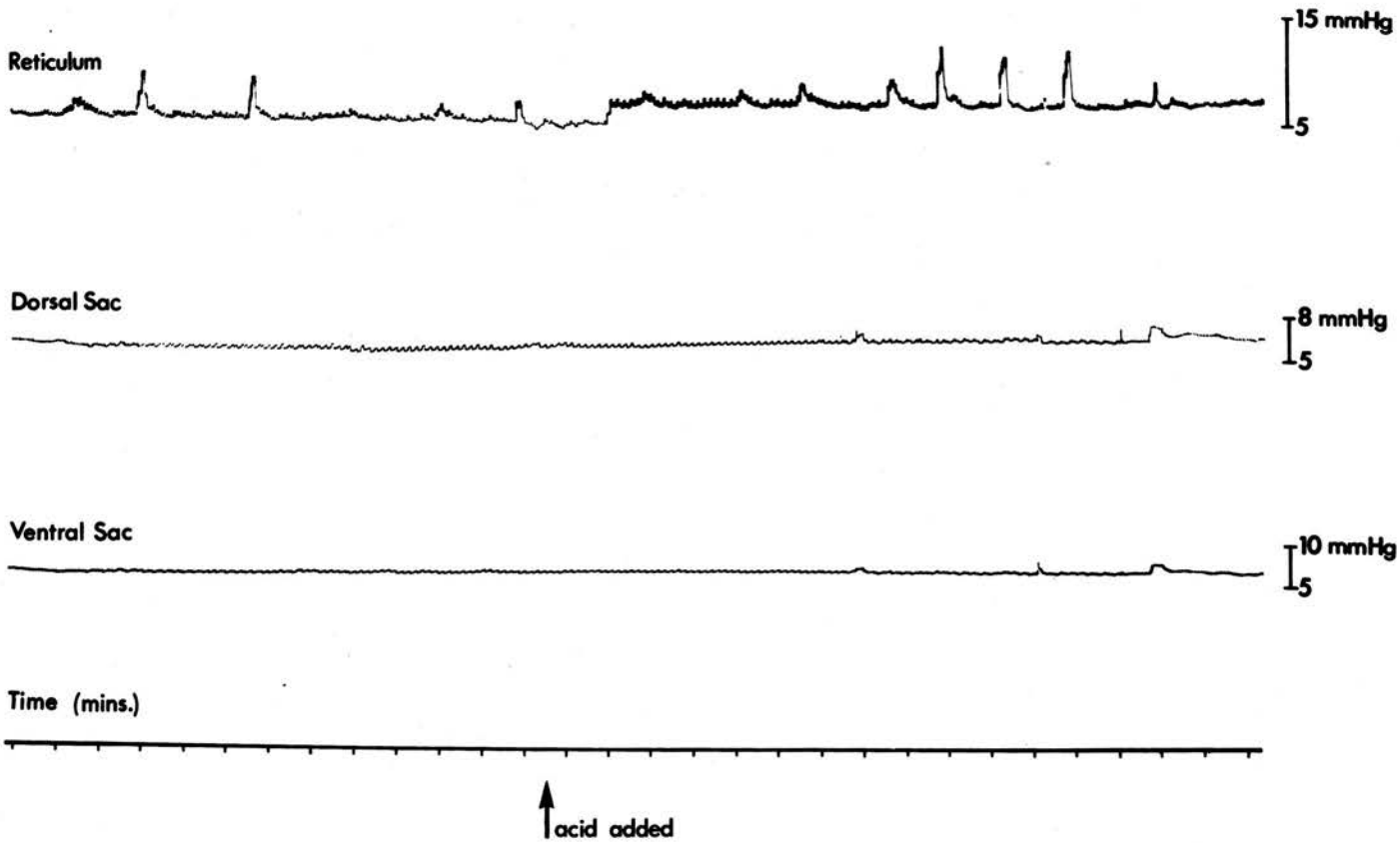

Reticulum
17 mmHg
7
Dorsal Sac
10 mmHg
5
Ventral Sac
10 mmHg
5
Time (mins.)
Blood Pressure
90 mmHg
70
water added

Fig. 25. Pen recorder tracing showing the reappearance of rhythmic contractions of the reticulum (upper) on addition of 100 ml. N/10 hydrochloric acid to the abomasum (arrow). This procedure lowered the pH of the abomasal contents from 2.2 to 1.6. The dorsal and ventral sacs of the rumen show only very infrequent contractions.

Decerebrate Blackface sheep, 1 yr. old.


Reticulum
15 mmHg
5
Dorsal Sac
8 mmHg
5
Ventral Sac
10 mmHg
5
Time (mins.)
acid added

Another factor which appeared to influence gastric motility in the decerebrate animal was the state of the respiratory system. On several occasions slight hyperventilation of an animal with the Palmer Respiration Pump was followed by the appearance of rhythmic contractions of the reticulum where these had been absent previously; this was observed, even if the sheep had been breathing spontaneously before hyperventilation. Contractions also tended to disappear if carbon dioxide levels were allowed to increase during closed circuit anaesthesia; in such cases, renewal of the soda lime led to the re-establishment of motility.

In one experiment, in which contractions of the reticulum were occurring rhythmically, periodic respiration appeared; after a series of 8 to 10 deep breaths, respiration was suspended for up to 80 sec. The rhythmical pattern of reticular motility at once disappeared, and was replaced by a motility pattern in which the reticulum consistently contracted immediately after each group of respiratory movements (Fig. 26). This sequence was observed over a period of about 15 minutes; artificial ventilation was then established, and the earlier rhythm of contraction was restored. Careful control of ventilation appears to be of great importance.

One rather surprising finding was that motility was never evoked in animals with a blood pressure over 85mm. Hg. Mean diastolic blood pressure was usually 70 to 80 mm. Hg. following decerebration, but in five animals values of 100 to 110 mm. Hg. were observed following the initial fluctuations induced by removal of the cerebrum and cerebellum. Rhythmical contractions of the rumino-reticulum could not be elicited in such animals by distension of the reticulum or acidification of the contents of the abomasum although when blood pressure had fallen below about 80 mm. Hg. such procedures readily evoked

Fig. 26. Pen-recorder tracing showing reticular motility during periodic respiration in a decerebrate Blackface sheep. The gain on the tracing from the ventral sac of the rumen has been increased to show the periods of respiration. Immediately following each period of ventilation, there is a small contraction of the reticulum (upper trace).

The dorsal sac of the rumen is not contracting.

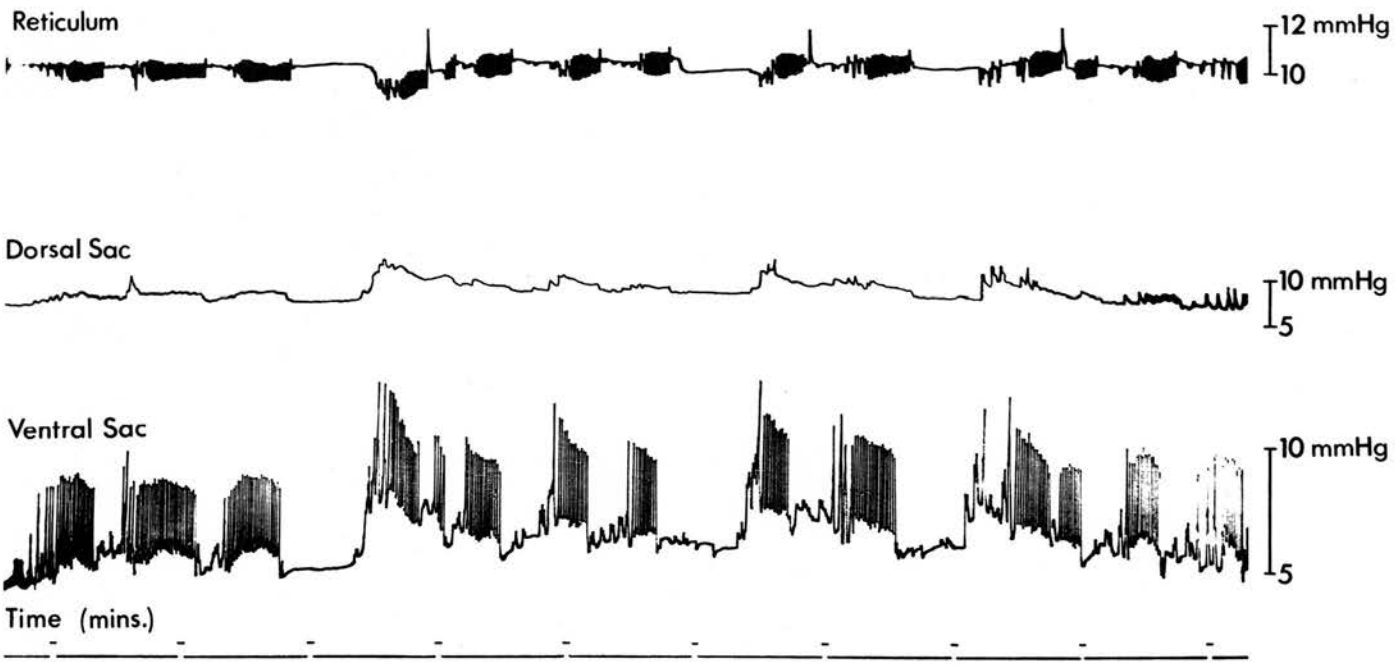
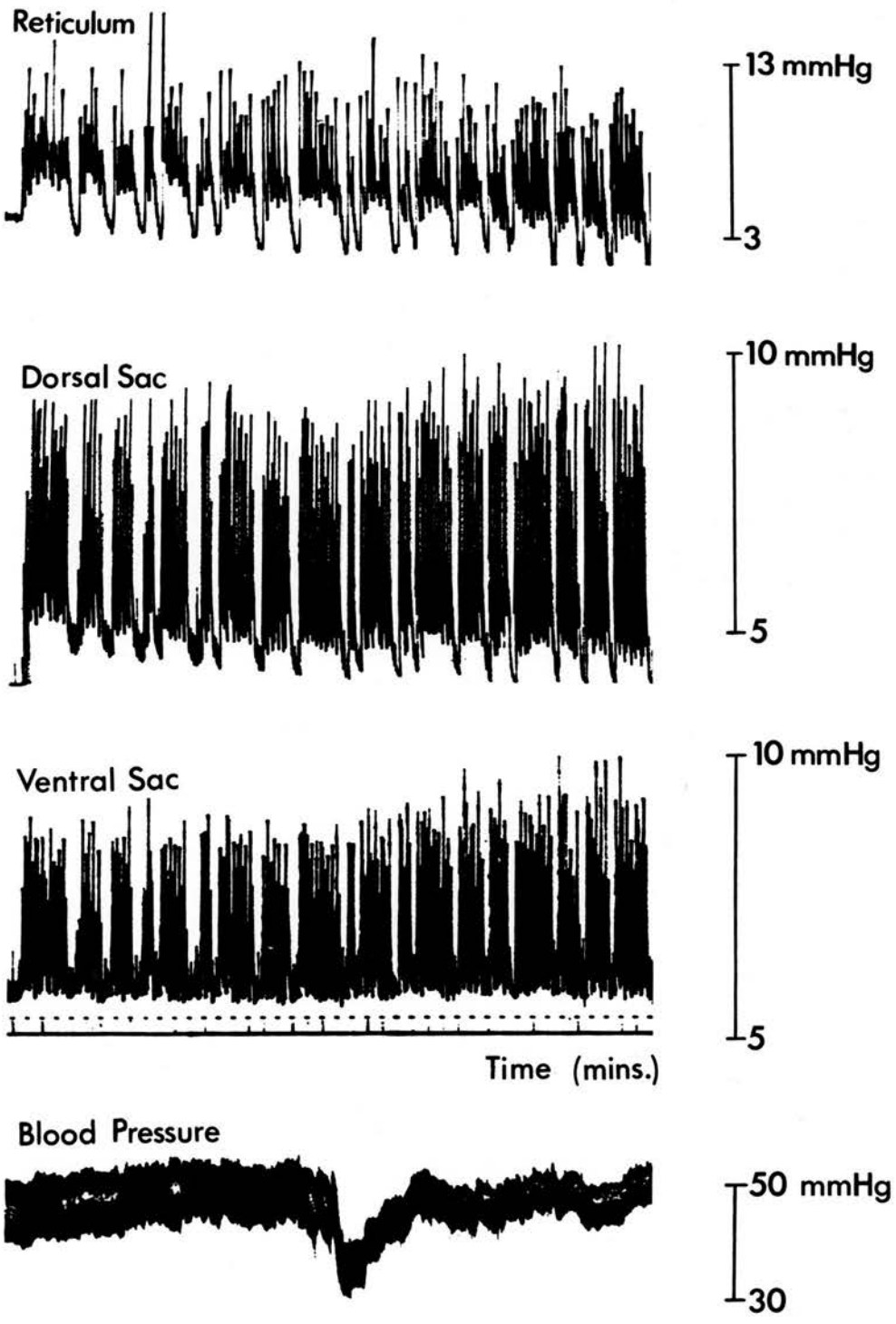

Reticulum
12 mmHg
10
Dorsal Sac
10 mmHg
5
Ventral Sac
10 mmHg
5
Time (mins.)

Fig. 27. Pen recording showing the irregular pattern of motility which was frequently recorded from the forestomach of decerebrate sheep when the blood pressure had fallen below about 50 mm. Hg. Large amplitude contractions occur as synchronised bursts in the reticulum and rumen, separated by only very short intervals of quiescence.


Reticulum
13 mmHg
3
Dorsal Sac
10 mmHg
5
Ventral Sac
10 mmHg
5
Time (mins.)
Blood Pressure
50 mmHg
30

a series of contractions. On the other hand, rhythmic gastric contractions were often recorded when the blood pressure was 30 mm. Hg. or less (all data presented here were obtained from animals with a mean diastolic blood pressure of 50 mm. Hg. or more). Under such circumstances, the frequency of reticular contractions was often very high - up to four per minute - and each pressure event showed several phases (see Fig. 27). One animal in which this type of motility was observed continued to exhibit the pattern for 8 hr., while the blood pressure gradually declined from 45 to 30 mm. Hg. when the animal was destroyed - 21 hr. after decerebration.

Electrical Stimulation of the Medulla Oblongata

Monopolar stimulation was carried out using Wood's - metal - filled glass electrodes of tip diameter about 0.1 mm., with a thin covering of platinum plated over the tip surface; these had an 'in situ' D.C. resistance of 0.2 to 0.3 MΩ. Alternatively, silver wire electrodes, the shanks of which were insultated with 'Insulex', were used; these had a tip diameter of 0.5 mm. and the tip length of about 1 mm. For stimulation of the entire length of the dorsal vagal nucleus anterior to the obex, a silver wire of 0.5 mm. diameter was shaped to run along the course of this, and lowered into position on the floor of the IVth ventricle, above this part of the nucleus. The indifferent electrode in each case was a brass slug located in the rumen contents. Stimuli were delivered from a Grass SD5 isolated stimulator, as biphasic pulses of 5 msec. duration and 50 c.p.s., at a series of voltages for each electrode position (see Fig. 23).

Electrolytic lesions were made after each point in the medulla had been examined, by passing a D.C. current of about 1 mA. for 10 sec. Histology was routinely carried out after formalin fixation of the brain stem, by cutting transverse sections of the

medulla oblongata on a Reichert freezing microtome, type OmE, at 100 μ, staining for 1 to 2 min. with 1% Toluidin blue. After differentiation for 1 to 2 min. with 95% alcohol, and immersion for 30 sec. in absolute alcohol, sections were cleared in cedarwood oil, and the positions of electrode tracts and principal nuclei were sketched under the camera lucida.

Electrophysiological Arrangement for Recording Unit Activity

Micro-electrodes

Micro-electrodes were prepared by a modification of the method proposed by Gesteland, Howland, Lettvin and Pitts (1959) as follows:-

> Glass electrodes (5 cm. long) are prepared with a double-pull solenoid-operated electrode puller, and the tips of these are broken, under a microcope with a graticule eyepiece, to give a diameter of about 5 μ. An alloy of two parts Wood's metal to one part Indium is just fused, and drawn up into polythene tubing, the interior diameter of which is very slightly less than that of the glass electrodes. The polythene is now cut free, and the alloy wire cut into lengths of about 2 cm.; one such length is inserted into the shank of a micro-electrode, and pushed down to the neck in the glass by means of a length of copper wire (5 cm.) chosen to give as close a fit to the glass as possible. By holding the neck of the micro-electrode 2 to 3 cm. above a soldering iron, the Wood's metal is allowed to just melt, and is then forced to the tip using the copper wire as a plunger. Heat from the same source is used to fuse the copper to the alloy. providing a low noise, low impedance electrical connection. Finally, by gently warming the extruded part of the electrode, a small ball of Wood's metal is made to appear at the tip, removing any air bubbles which may have been left; this ball was dislodged by stroking the electrode tip against hairs on the back of the hand. Immediately before use, a black platinum tip is coated onto the micro-electrode in the manner described by Gesteland et al (1959).

Fig. 28. Tip of micro-electrode seen under the microscope. The metal filling makes the tip appear larger than it actually is (about 10μ). Note that the taper is very pronounced - electrodes with this characteristic tended to be most satisfactory.

Micro-electrodes prepared in this way have an 'in situ' D.C. impedance of 1 to 2 MΩ, and while having electrical properties superior to those of tungsten, they are sufficiently robust to withstand the mechanical stresses imposed by pulsation of the brain stem in time with the pulse and respiration (see Fig. 28).

Recording apparatus.

When the sheep had been prepared as outlined earlier, the screening cage of fine mesh, perforated zinc, was placed over the animal; a small door in this provided access to the recording site, so that the electrode manipulator could be controlled from outside the screen. The animal earth consisted of a silver wire, coated with a thin layer of silver chloride, which was inserted subcutaneously on the dorsal aspect of the cranium. Occasionally a second animal earth (a brass slug, located in the rumen contents) was required, to prevent interference being picked up by the remainder of the sheep's body.

The output from the micro-electrode was fed into a Bak standard Wide Band Electrometer via a calibration box and was subsequently amplified by a Tektronix 122 Preamplifier, before being displayed through a 2A63 channel of a Tektronix 565 dual beam oscilloscope. The time-constant of this system was 25 msec. The other beam of the oscilloscope was chopped with a 3A72 chopper unit; it displayed the output from a 1 sec. time marker (Venner type TSA 602/A) and the preamplifier output from any one channel of the Devices M8 pen recorder. This arrangement made it possible to compare unit activity recorded from the micro-electrode with any of the traces displayed on the pen recorder (Fig. 29).

A Cossor model 1458 camera with Cossor 1431 drive unit was used in conjunction with the oscilloscope to photograph spike activity on Kodak R55 recording film; an electrical circuit was

Fig. 29. Block diagram of the electro-physiological apparatus for recording unit activity from the dorsal nucleus of the vagus.

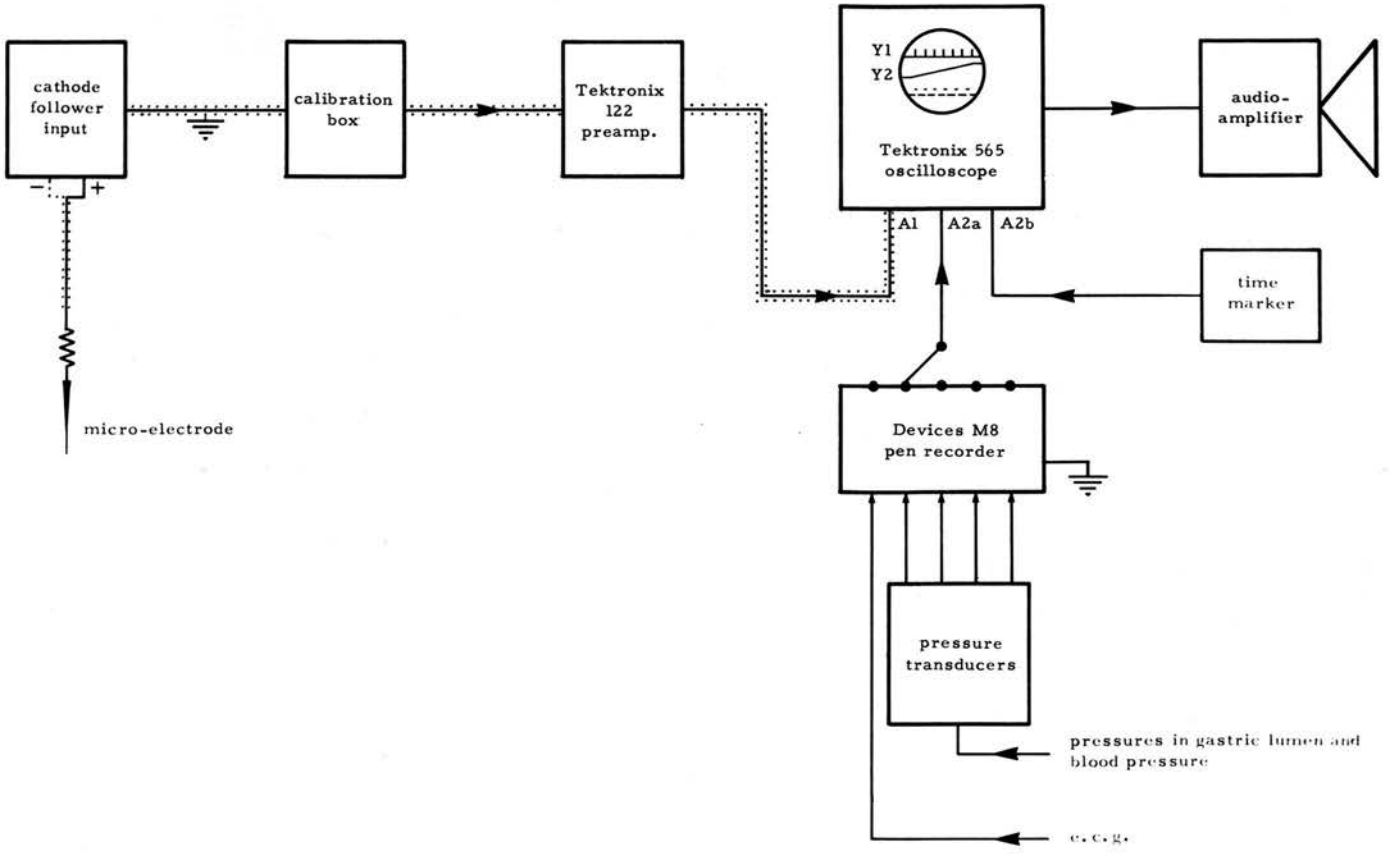
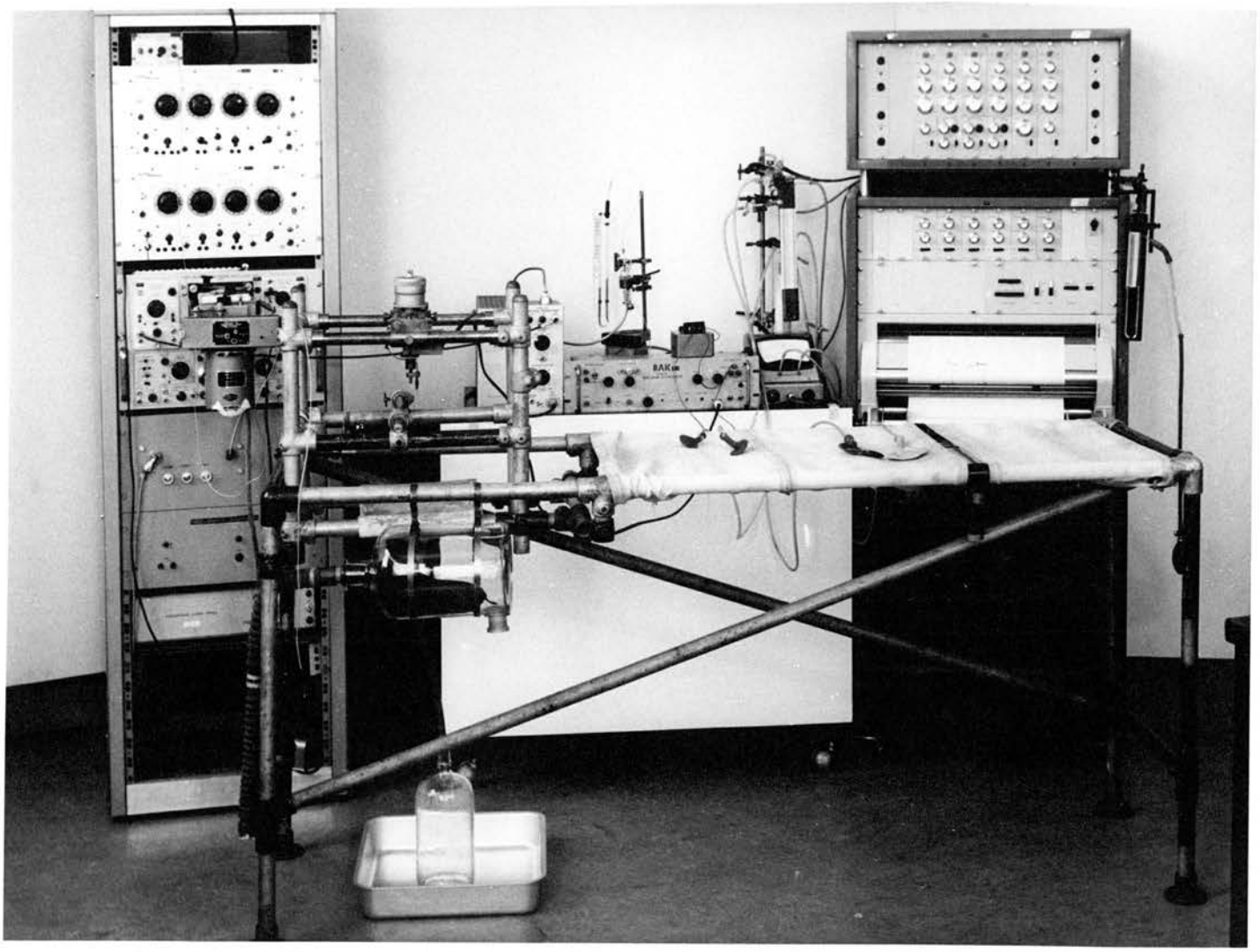

cathode follower input
calibration box
Tektronix 122 preamp.
Y1
Y2
Tektronix 565 oscilloscope
audio-amplifier
A1
A2a
A2b
time marker
micro-electrode
Devices M8 pen recorder
pressure transducers
pressures in gastric lumen and blood pressure
e.c.g.

Plate 1. Apparatus used for recording unit activity from the medulla oblongata. The stereotaxic frame is in front of the electronic recording apparatus, and shows the method used to 'sling' the sheep on a canvas hammock, with the head located at the ears, lower orbital margin, hard palate, and bridge of the nose.



established via a relay, so that opening the shutter of the camera automatically disconnected the time base and applied a standing voltage to the X plates of the oscilloscope, returning the beams to the centre of the screen; a marker on the pen recorder was also activated, to indicate the position at which the photograph had been taken.

After a recording from each site, lesions were prepared as described following electrical stimulation, and histology was routinely carried out to confirm the position of electrode stabs, as described in the section on electrical stimulation.

RESULTS

THE DISTRIBUTION OF NEURONS SHOWING CHROMATOLYSIS

All animals for which data are presented remained in good health, and after some depression of appetite lasting 1 to 3 days appeared to feed normally.

Post-mortem examination of all sheep established that vagal transection was complete, except that in one animal (A4) the lesion involved a thick strand of connective tissue; the nerve remained intact alongside this; this particular sheep is therefore treated as a control animal, together with one other which was subjected to thoracotomy, although no attempt was made to cut the vagus. Portions of the nerve trunk distal to the lesion were fixed and stained in osmic acid, and transverse sections prepared in the manner described in section 3. Myelinated axons were not present, although globules of fat stained with osmium were distributed unevenly along the course of the nerve.

The distribution of the vagal trunks is described in detail in section 3; on gross examination, no anastomosis was found between the dorsal and ventral trunks, posterior to the level of section.

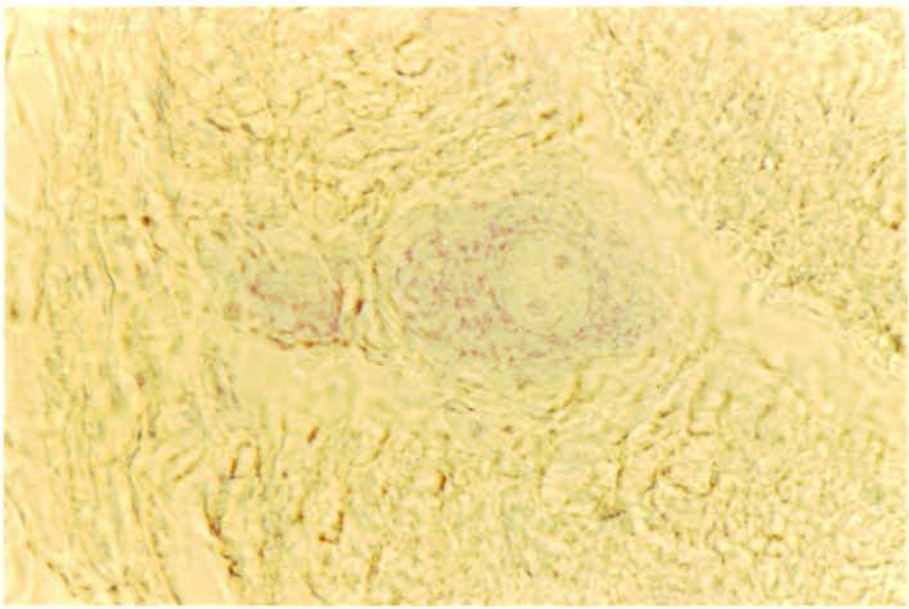
After vagal transection, neuron degeneration was always present in the dorsal nucleus and inferior ganglia of the vagus nerve. In general, cells showing chromatolysis stained much less intensely than normal cells (see Fig. 30) due to loss of Nissl substance, and were easily recognised by the consequent loss of cell outline.

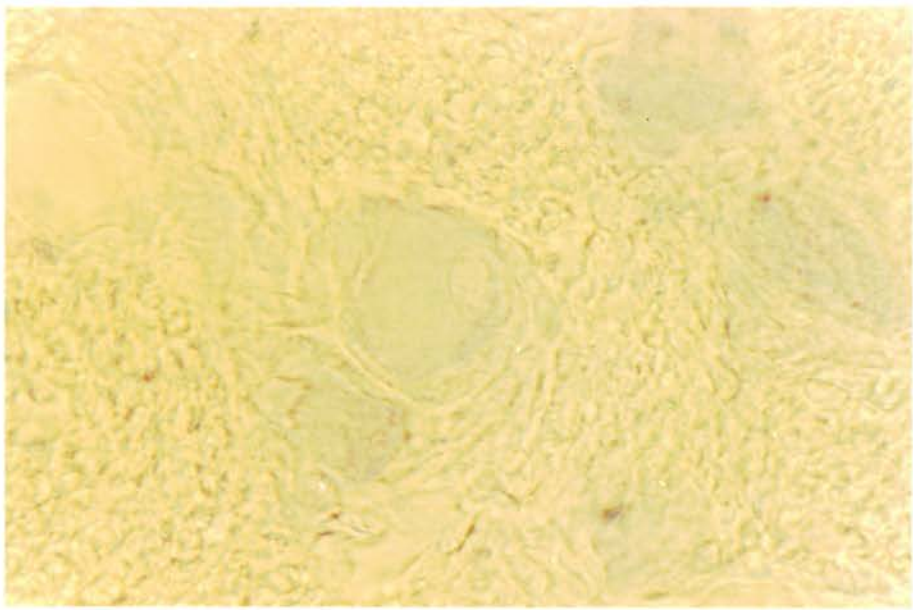
Occasionally, cells showing the morphological features of chromatolysis were seen in other parts of the brain stem, particularly in the reticular formation of the medulla, and those parts of the dorsal nucleus of the vagus lying posterior to the

Fig. 30. Cells from the dorsal nucleus of the vagus. Sheep was subjected to dorsal vagotomy, and 14 days later sacrificed. Serial sections of the medulla were stained with toluidin blue.

Upper - normal

Lower - chromatolysis





obex. Similar features were also seen in preparations taken from animals in which neither vagal trunk had been severed, and so it is assumed that such changes are not a result of vagotomy. This 'background' pattern of Nissl lysis and cell swelling was sufficient to account for all cells showing such changes in areas other than those in the dorsal vagal nucleus and inferior ganglion.

After division of the dorsal vagal trunk, degenerative changes were seen in the dorsal nucleus and inferior ganglion of the vagus on both sides, although degeneration was most marked on the left, especially in the inferior ganglion, in which three times as many degenerating cells were found on the left sides as on the right (see Fig.31). In the dorsal vagal nucleus this inequality of distribution was less apparent; the nucleus of the left side contained about 50% more degenerating neurons than the nucleus on the right (see Fig.32). Within the dorsal nucleus of the vagus degenerating cells were confined almost exclusively to that part anterior to the obex, although within this area the number of degenerating cells seen on each section was fairly constant, except at the extreme anterior extremity where there are few neurons in the nucleus. However, because of the greater total numbers of cells in the anterior third of the nucleus, the greatest percentage of degenerating nerve cells occurred in the 1 mm. length of the nucleus lying immediately anterior to the obex.

Section of the ventral vagal trunk, on the whole, caused a similar pattern of degeneration to appear, but with a preponderance of cells showing chromatolysis on the right side. Fewer cells were involved, however, so that only 50% of the cells of the inferior ganglion of the vagus on the right side had degenerated, and about 20% of the cells in the left ganglion (see Fig.33). Degeneration was also less marked in the

Fig. 31. The distribution of normal (clear outline) and degenerating (shaded) cells in the superior and inferior ganglia of the vagus, 14 days after transection of the dorsal abdominal vagal trunk. Mean data from 2 animals.

Upper - right vagus
Lower - left vagus
Centre - contour of a longitudinal section of the vagus, extreme upper extremity to the left. Distances in mm. shown on lower scale.

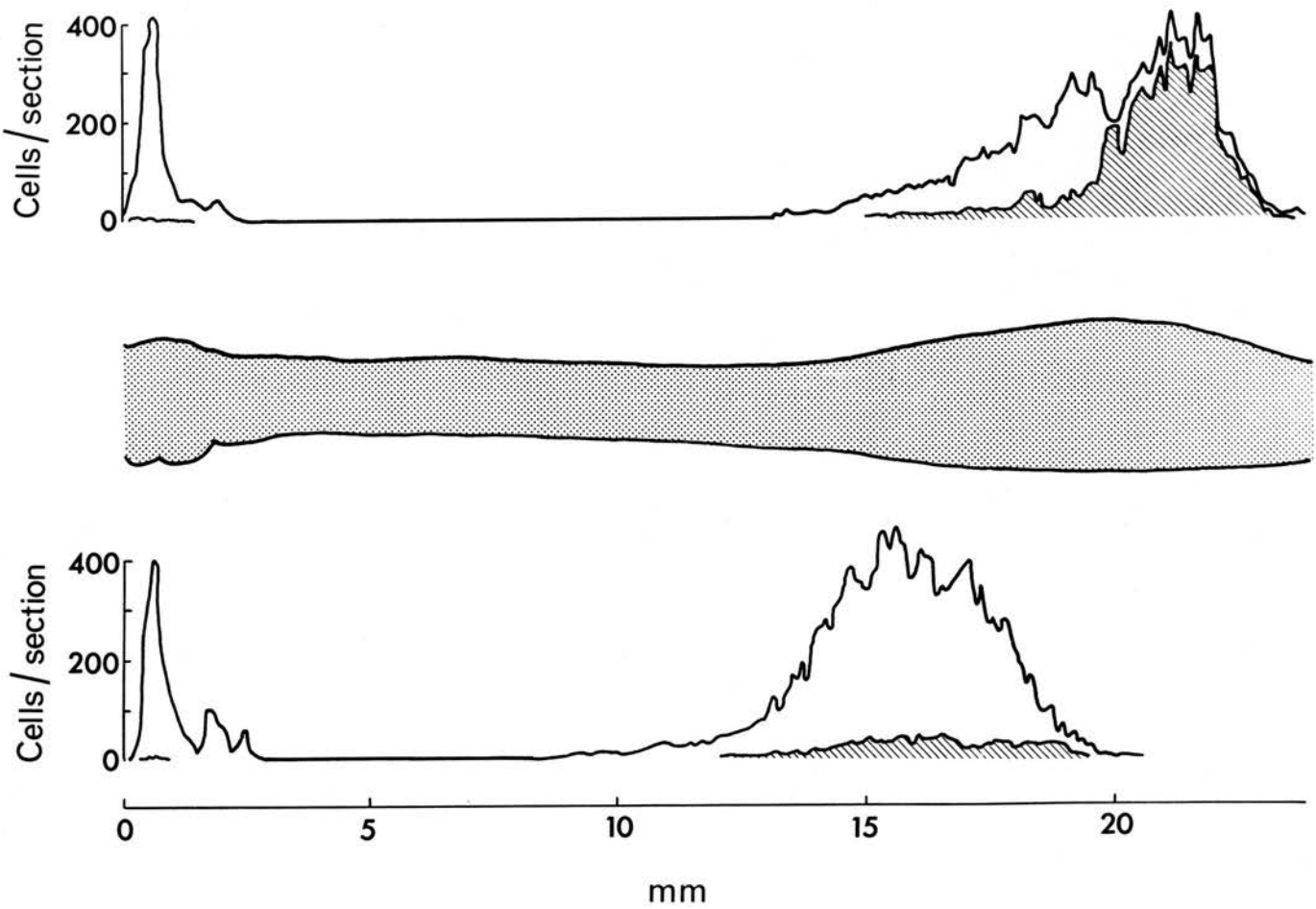

Cells / section
400
200
0
Cells / section
400
200
0
0
5
10
15
20
mm

Fig. 32. The distribution of normal (clear outline) and degenerating (shaded) cells in the dorsal vagal nuclei, 14 days after transection of the dorsal abdominal vagal trunk. Mean data from 2 animals.

Upper - left nucleus

Lower - right nucleus

Centre - longitudinal horizontal section of the medulla, drawn to the same scale, showing the nuclei in plan. Scale in mm. relative to the obex shown below.

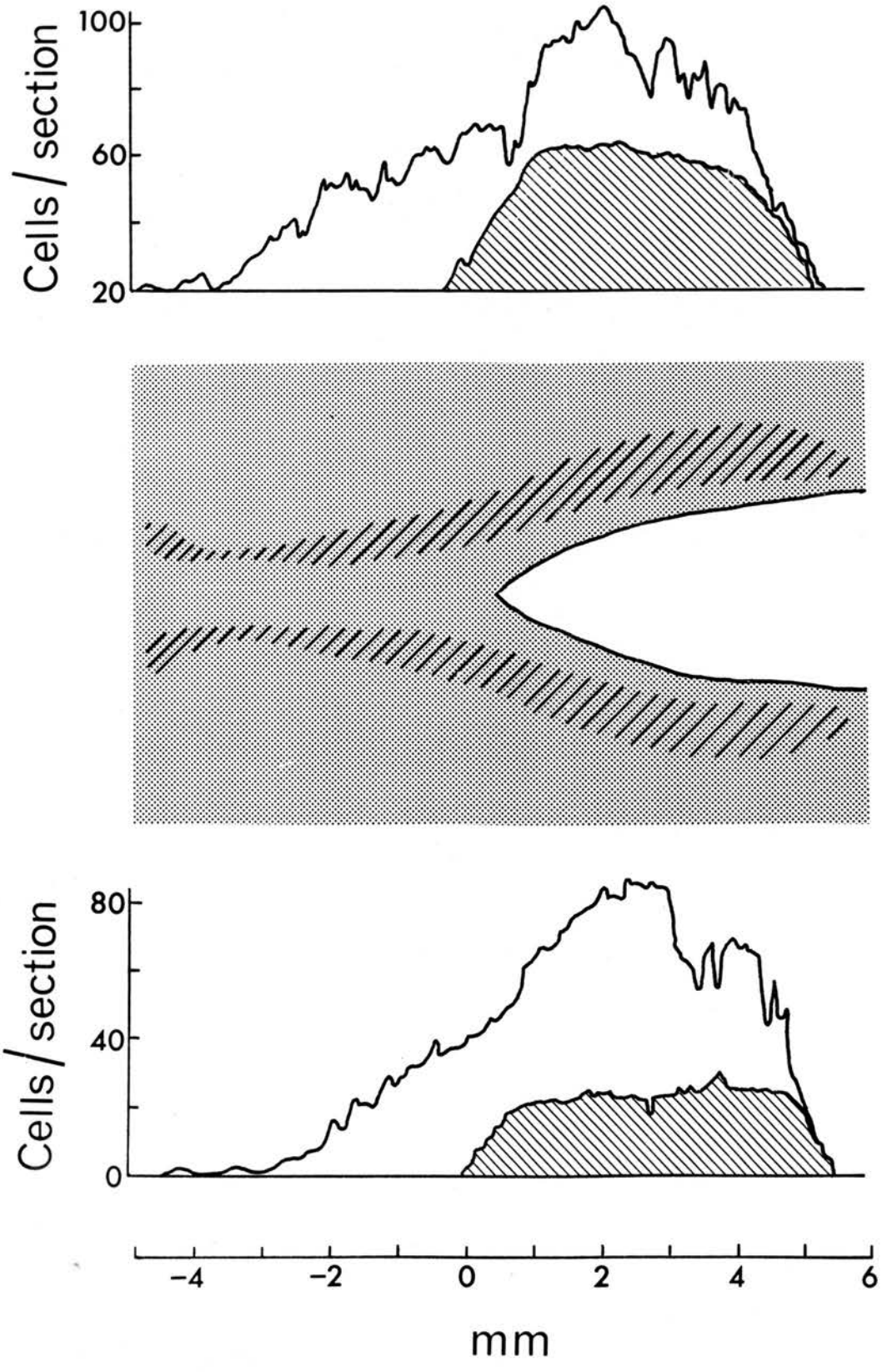

Cells / section
100
60
20
Cells / section
80
40
0
−4
−2
0
2
4
6
mm

Fig. 33. The distribution of normal (clear outline) and degenerating (shaded) cells in the superior and inferior ganglia of the vagus, 14 days after transection of the ventral abdominal vagal trunk. Mean data from 2 animals.

Upper - right vagus
Lower - left vagus
Centre - contour of a longitudinal section of the vagus - extreme upper extremity to the left. Distances in mm. shown on lower scale.

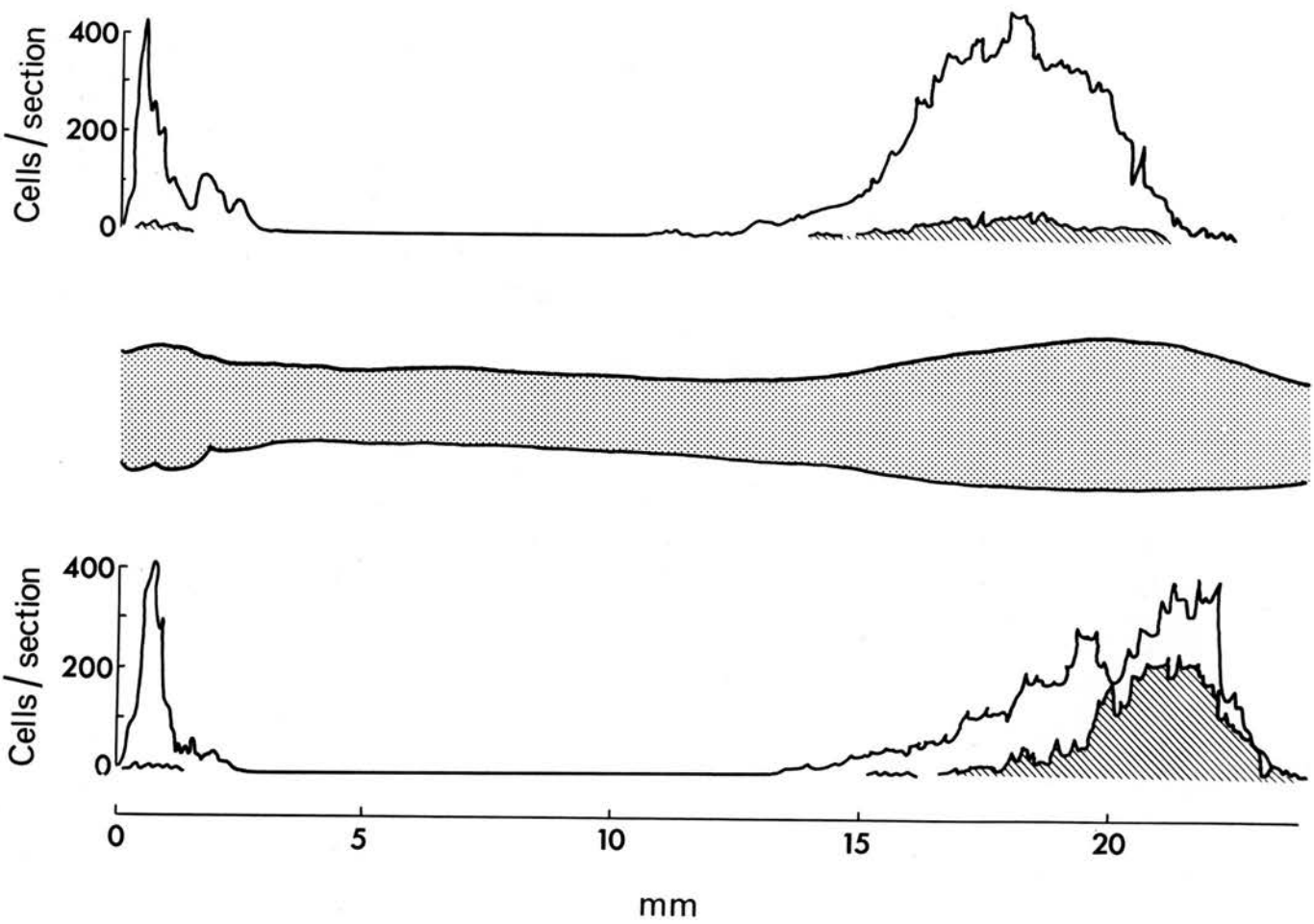
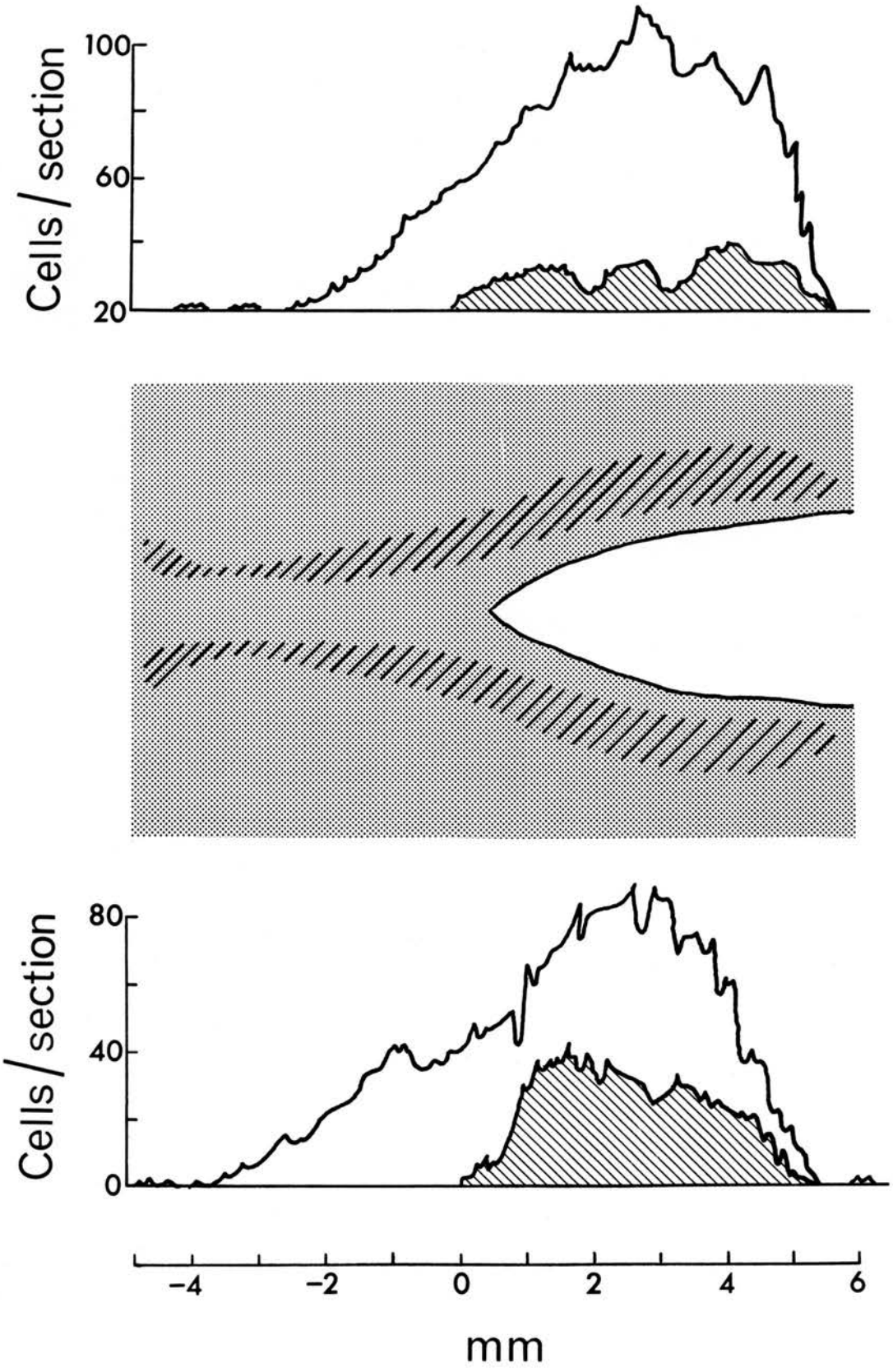

Cells / section
400
200
0
Cells / section
400
200
0
0
5
10
15
20
mm

Fig. 34. The distribution of normal (clear outline) and degenerating (shaded) cells in the dorsal vagal nuclei, 14 days after transection of the ventral abdominal vagal trunk. Mean data from 2 animals.

Upper - left nucleus
Lower - right nucleus
Centre - longitudinal horizontal section of the medulla drawn to the same scale, showing the nuclei in plan. Scale in mm. relative to the obex shown below.


Cells / section
100
60
20
Cells / section
80
40
0
−4
−2
0
2
4
6
mm

dorsal nucleus than it had been following section of the dorsal vagal trunk.

Systematic investigations of the pons and of the hypothalamus failed to reveal any further areas of cell degeneration following vagal section. Other areas of the brain stem were not fully examined.

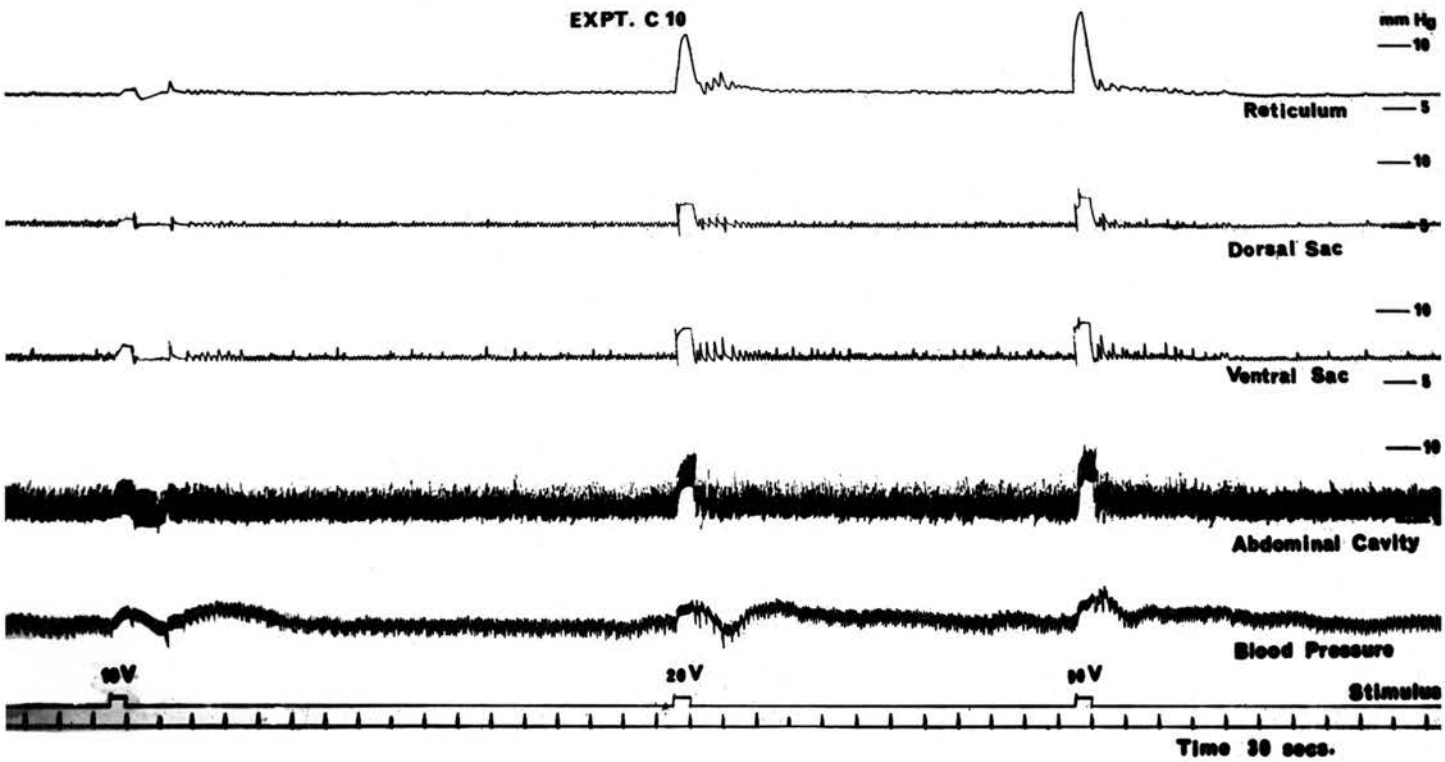
Electrical Stimulation of the Medulla Oblongata.

During these experiments a total of 30 sheep were used, and an average of 20-30 loci were examined in each experimental animal. The dorsal aspect of the medulla was explored by punctate monopolar electrical stimulation with biphasic pulses of 5 msec. duration at 4 --40 c.p.s. and up to 30V intensity for periods of up to 20 secs.

In a preliminary investigation involving 8 sheep, none of which was showing spontaneous gastric motility, contraction of the rumino-reticulum was elicited from only 2 loci in 2 animals(in the remaining 22 experiments, when the forestomach was contracting rhythmically, focal stimulation using these stimulus parameters failed to evoke contraction). On these two occasions, the forestomachs showed no spontaneous motility, but on electrical stimulation, monophasic contractions of the reticulum occurred (Fig. 35) after a latency of about 1 sec. and 20 secs. respectively. In the latter case, stimulation at a strength of 30V was applied about 1 min. after an isolated, very low amplitude contraction of the rumen; after a latency of about 20 secs. the reticulum contracted strongly (15 mm.Hg. pressure) for 6 secs., and relaxed quickly. This was repeated 12 mins. later and again after 15 mins. - there was no spontaneous motility of the reticulum, although the contractions of the rumen increased progressively in amplitude up to 5 mm.Hg., occurring at a frequency of about 1/5 mins., each contraction lasting 30-40 secs. The appearance of motility in the rumen does not, therefore, seem to have been caused by stimulation, although contractions of the reticulum were. This response was obtained when the electrode tip was situated 8 mm. behind the obex, 1 mm. lateral to midline and 3 mm. deep.

The other point from which contraction of the reticulum could be elicited was situated 6 mm. behind the obex, 1 mm. lateral to midline and 3 mm. deep - from here, point electrical stimulation (at 20-30V) evoked monophasic contractions (5 mm.Hg. in amplitude) of the reticulum lasting 30 secs. The rumen did not contract during this experiment. Gastric responses occurred independently of marked drops in blood pressure, and

Fig. 35. Kymograph tracing showing an excitatory response to stimulation of the medulla oblongata. The electrode tip was lying 6 mm. posterior to the obex, 1 mm. lateral to midline and 3 mm. deep. Increasing strengths of stimulation were applied at the points shown, for 15 secs. in each case. On each occasion, the sheep responded by stiffening along the spinal axis, extension of the limbs, and tensing of the abdominal muscles; this latter feature has caused a simultaneous increase in pressure within the abdominal cavity and rumen, as can be seen from the corresponding traces. Respiration was suspended during stimulation, and was slower immediately afterward. Blood pressure fluctuated irregularly. The rise in pressure within the reticulum on stimulation at 20 V and 30 V is much more marked than pressure rises at other points within the abdomen.


EXPT. C 10
mm Hg
10
Reticulum
5
10
0
Dorsal Sac
10
Ventral Sac
5
10
Abdominal Cavity
Blood Pressure
10V
20V
30V
Stimulus
Time 30 secs.

in the second instance cited the immediate response was a small rise in blood pressure associated with an increase in heart rate from 48 to 50 / min. (Fig. 35).

When the tip of the electrode was lowered deeper into the reticular formation, it was found that at certain positions electrical stimulation consistently caused contractions of the forestomach. These closely resembled the contractions seen following electrical stimulation of the cervical vagi, or of the dorsal thoracic vagus. Thus the pressure rise was first noticed in the reticulum, and was closely followed by increases in pressure in both dorsal and ventral sacs of the rumen. On prolonged stimulation, the contraction of the reticulum showed a marked fall in amplitude after 5 - 6 secs. whereas dorsal and ventral rumen sacs remained in a state of contraction for 40 secs. or more. In two experiments in which recording balloons were placed in the anterior and posterior parts of both dorsal and ventral sacs, the increase in pressure occurred simultaneously at all four points in the rumen. Histological controls established that responses of this type were elicited from those parts of the medulla through which fibres passed between the dorsal vagal nucleus, and the vagal rootlets on the ventro-lateral aspect of the medulla oblongata. Often, following the initial response at such points, there occurred a series of 2 - 4 contractions of the reticulum each lasting 10 secs. and occurring within 12 secs. of the end of the previous contraction. These had an amplitude of about one third that induced initially, and fell off gradually (see Fig. 39). Motility of this type, continuing for several seconds after the cessation of stimulation, is probably reflex in nature, and resembled that described by Dussardier (1960).

Monopolar stimulation at higher voltages (up to 100V) caused contraction of the reticulum and dorsal and ventral sacs of the

rumen, these occurring almost simultaneously, and resembling those seen on direct efferent vagal stimulation (see section 2). Such responses could be elicited from anywhere on the dorsal surface of the medulla, by suitably adjusting the voltage of stimulation, and were usually accompanied by extension of the limbs, tensing of abdominal muscles and stiffening along the spinal axis. Delayed reticular or ruminal responses were not seen under such conditions.

These initial experiments, involving 8 sheep, were carried out on both anaesthetised (Halothane) and decerebrate animals, and the results obtained in both cases were identical. The effects of the anaesthetic could not, therefore, have been responsible for failure to establish gastric contraction by electrical stimulation of the dorsal nucleus of the vagus. Two possible explanations can be offered to explain these findings - either

1. the stimulus parameters did not excite a great enough number of motor neurons

or

2. there is, under these experimental conditions, a powerful inhibitory mechanism in the region of the dorsal vagal nucleus, which is either spontaneously active, or which becomes active when this part of the brain is electrically stimulated, and which can completely inhibit any excitatory effects established at the same time.

Two further experiments showed that electrical stimulation of the floor of the IVth ventricle, at a position corresponding to the course of the dorsal vagal nucleus, using an elongated silver electrode, shaped so that it ran longitudinally on the surface, failed to evoke any contraction of the rumen or reticulum unless the voltage was higher than 15V. When contractions did occur, they resembled those seen on high voltage electrical stimulation

of the dorsal aspect of the medulla as described above.

This finding fails to support the hypothesis that contractions were absent because insufficient numbers of cells had been excited by stimulation, and so provides indirect evidence for the second hypothesis. If such an inhibitory mechanism does exist, then it would be possible to demonstrate it by stimulation of the dorsal medulla during a reflexly evoked contraction of the reticulum. A further series of 20 experiments was therefore designed to test this point.

When the reticulum was distended by the introduction of warm water into the balloon in its lumen, and following lowering the pH of the abomasal contents to about 1.5, rhythmical contractions of the reticulum usually appeared at once, and were usually occurring regularly in 5 to 10 minutes. Under these conditions, the effects of point stimulation at low intensity were investigated. For this purpose, biphasic pulses of between 0.5 and 2 V were delivered for periods of 3 to 30 secs. (usually for 5 secs.), either just as the reticulum began to contract, or at some time before the reticulum was expected to be going to contract. Under these conditions, stimulation caused a reduction in amplitude of the reticular and ruminal contractions, and, less consistently, a marked but transient drop in frequency. These effects are shown in Figs. 36 and 37. The most satisfactory control in these experiments is the gastric motility present immediately before stimulation, as the precise pattern of contraction varies markedly during the 2 to 20 hr. of an experiment; all measurements given here are therefore related to this control. Usually, the reduction in amplitude of the reticular contraction was confined to that cycle and the succeeding contraction had an amplitude similar to the control. From certain positions, particularly those loci anterior to the dorsal nucleus of the vagus, which were found to modify rumino-reticular motility (see Fig. 38)

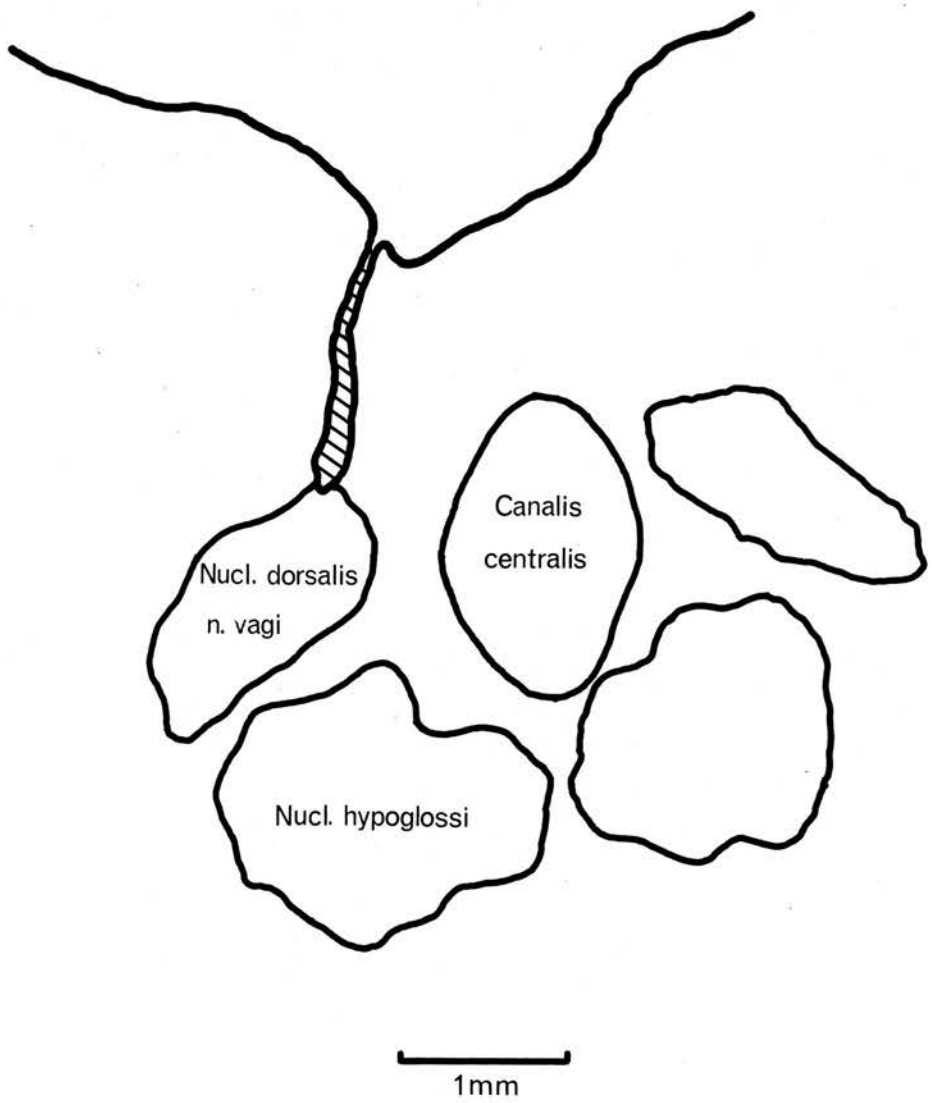


Fig. 36. The effect of electrical stimulation of the dorsal nucleus of the vagus at the point shown in above. The recordings of pressure within the reticulum and mid-dorsal and mid-ventral sacs of the rumen were made in the manner laid out in the text. Blood pressure was recorded from the anterior tibial artery via a G. E. C. strain guage transducer.

The amplitude of the 2nd phase of the contraction of the reticulum shows a marked diminution, and return to 'normal' occurs gradually over several motility cycles. At this position there is a slight drop in pulse rate and blood pressure associated with stimulation.

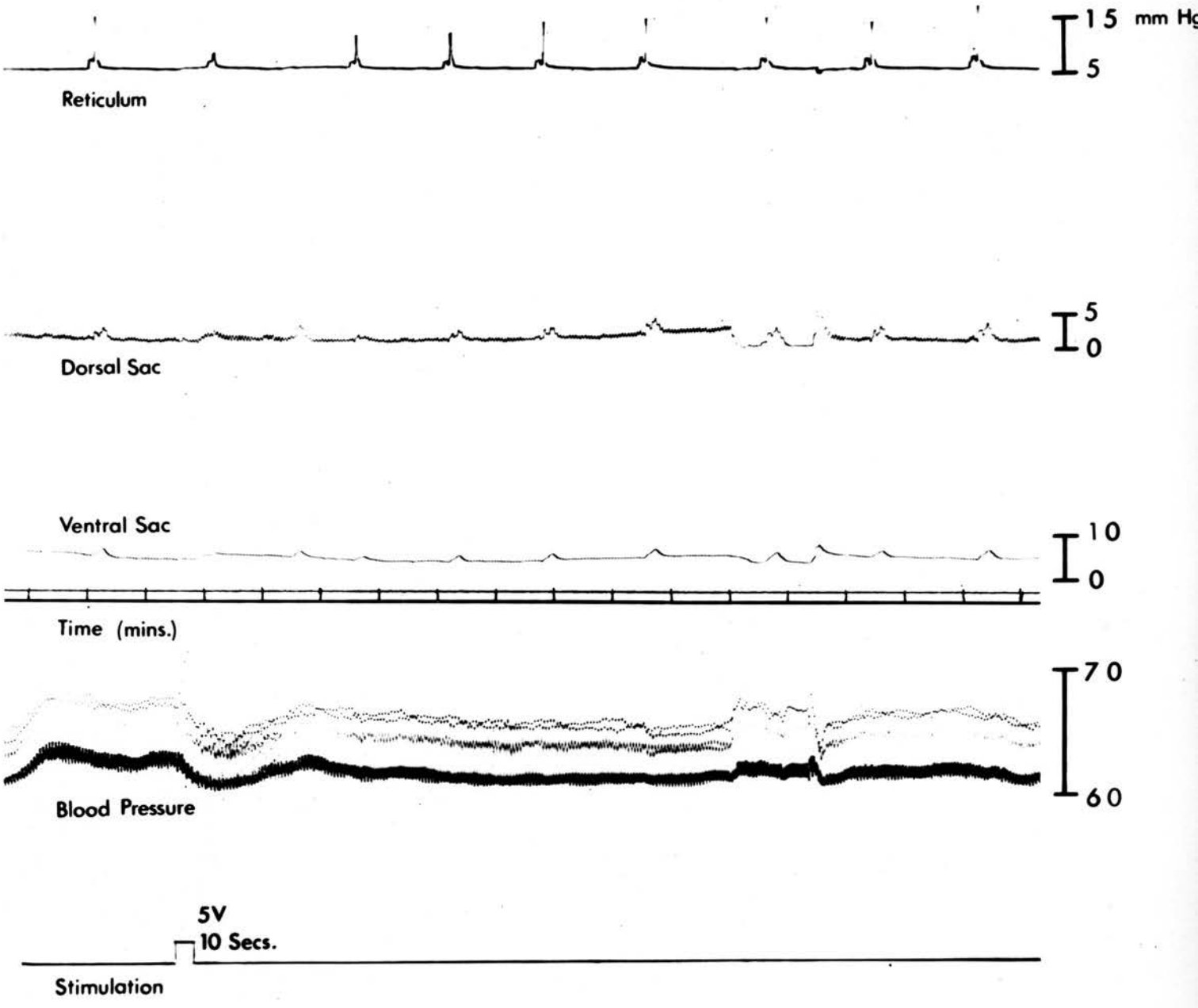
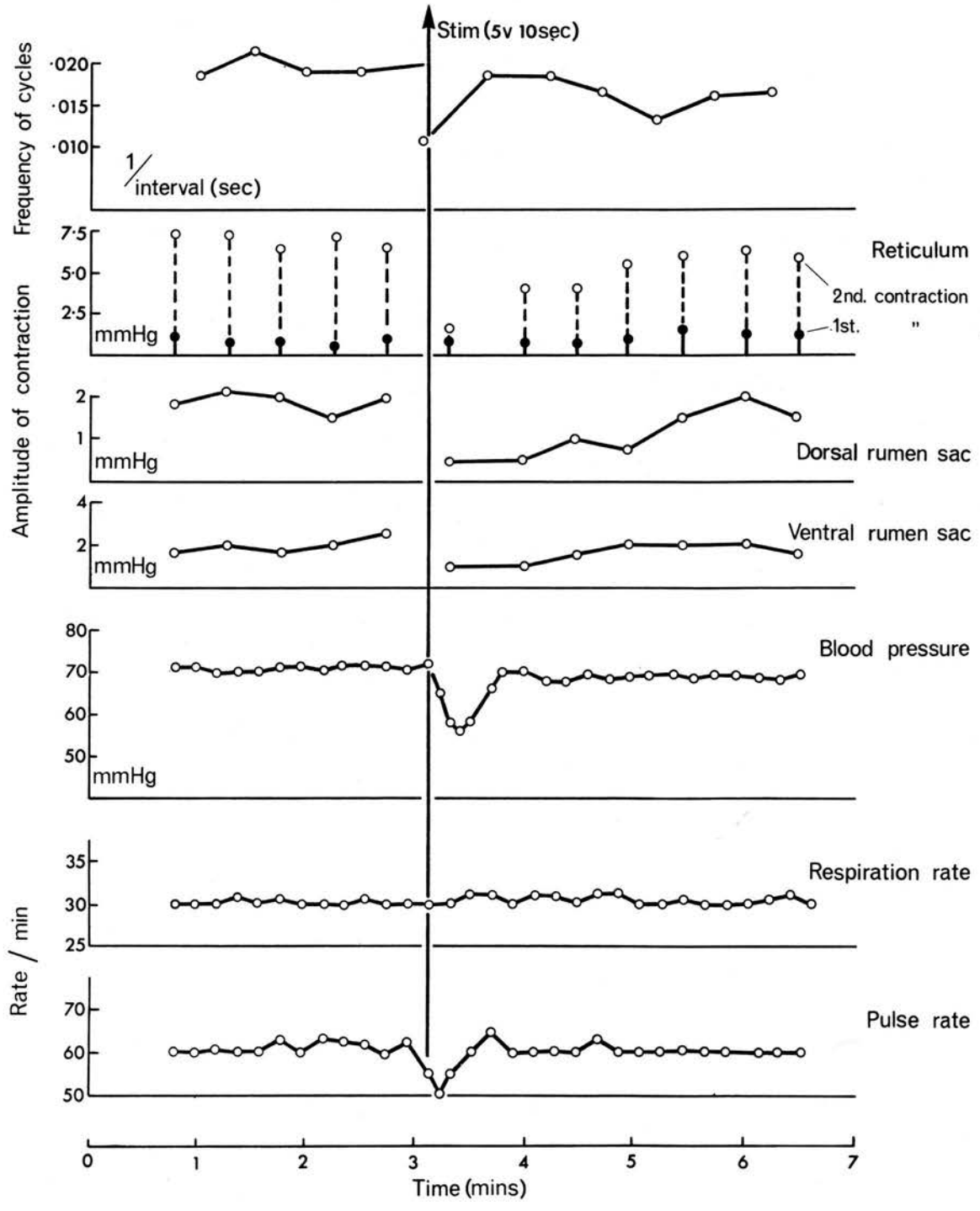

15 mm Hg
5
Reticulum
5
0
Dorsal Sac
Ventral Sac
10
0
Time (mins.)
70
60
Blood Pressure
5V
10 Secs.
Stimulation

Fig. 37. The response of the reticulum and dorsal and ventral sacs of the rumen to electrical stimulation at the point shown in Fig. 36. Depression of frequency is not particularly marked, but the great degree of inhibition of the 2nd reticular contraction persists over several cycles. Contraction of the dorsal and ventral sacs of the rumen is also depressed. Respiration is unaltered, although there is a brief drop in blood pressure associated with a fall in pulse rate. At more anterior levels the blood pressure and pulse rate were only rarely altered.


Stim (5v 10sec)
Frequency of cycles
·020
·015
·010
1/interval (sec)
Amplitude of contraction
7·5
5·0
2·5
mmHg
Reticulum
2nd. contraction
1st. "
2
1
mmHg
Dorsal rumen sac
4
2
mmHg
Ventral rumen sac
80
70
60
50
mmHg
Blood pressure
35
30
25
Respiration rate
Rate / min
70
60
50
Pulse rate
0
1
2
3
4
5
6
7
Time (mins)

the reduction in strength of the reticular contraction was prolonged over several cycles, returning to the previous level only very gradually. At all positions, however, it was found that the second phase of reticular contraction showed a much greater reduction in amplitude than did the first phase, as seen in Fig. 37.

Alterations in the rhythm of motility were less pronounced and less prolonged. However, when electrical stimulation was followed by a reduction in the frequency of contraction, the control rhythm was re-established without compensation for the imposed delay. There was thus no resemblance to the 'compensatory pause' exhibited by the heart as a result of electrical stimulation immediately before diastole. This point is shown more clearly in the diagram below, where each solid vertical stroke represents a contraction of the reticulum, and each arrow an electrical stimulus.

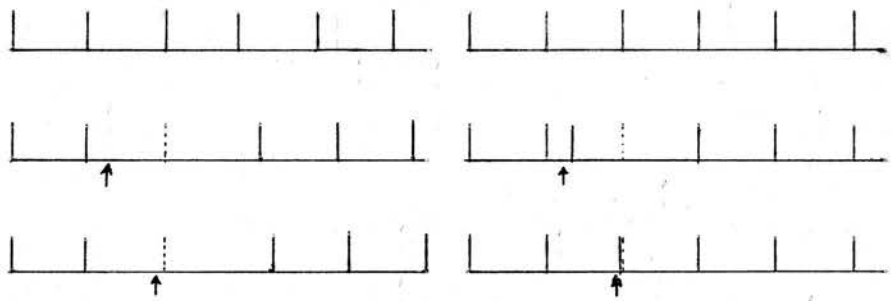
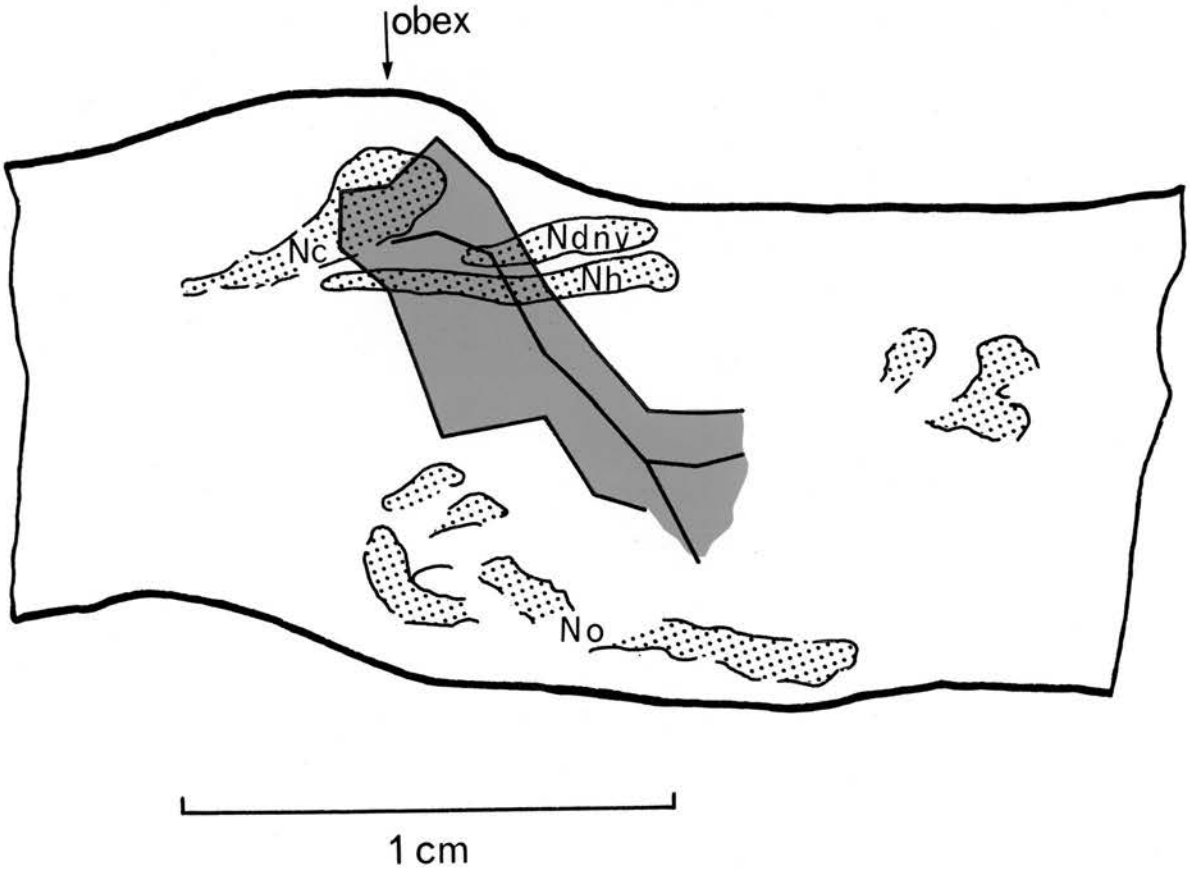


Fig. 38 shows a longitudinal section of the medulla, taken in a plane 2 mm. lateral to midline. This particular plane has been chosen because it was found, by the construction of contours on transverse sections, that maximum inhibitory responses were evoked from here. The main nuclear masses are outlined, and points of maximum suppression of the amplitude of reticular contractions following electrical stimulation, have been joined up to form a contour. It will be seen that areas from which most inhibition was obtained include the mid-part of the dorsal vagal nucleus, in the region of the obex, part of the area near the

Fig. 38. Location of the sites from which inhibitory responses were elicited. Longitudinal section of medulla oblongata cut 2 mm. lateral to mid-line.

Outline shows the zone from which the amplitude of gastric contractions could be depressed for 2 or more successive cycles by focal stimulation at 1 volt. Central line indicates region from which inhibitory responses were maximal, and reduction in amplitude of the succeeding contraction was at least 50 per cent.

Ndnv	Nucleus dorsalis N. vagi
Nh	Nuc. hypoglossi
Nc	Nuc. cuneatus
No	Nuc. olivaris

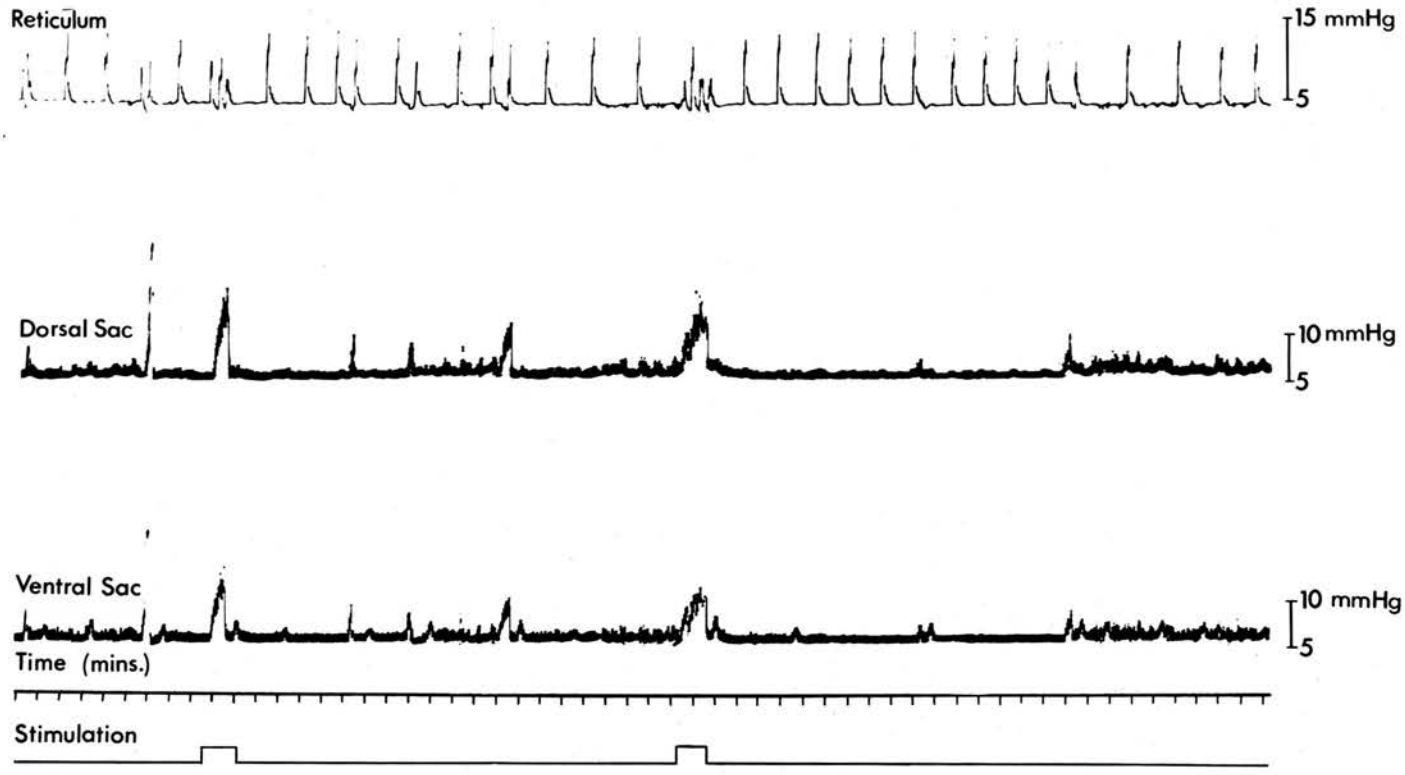

obex
Nc
Ndny
Nh
No
1 cm

nucleus ambiguus, and an oblique line connecting these two sites. A 'contour map' plotted in a similar manner, but relating to the effects of stimulation to the frequency of reticular contraction, showed a very similar distribution.

These effects could be elicited quite independently of alterations in blood pressure, pulse rate or respiration, although the former often showed a transient drop when the electrode tip was located in the dorsal motor nucleus of the vagus, particularly in the neighbourhood of the obex; when the blood pressure did fall, this was invariably associated with a drop in pulse rate (see e.g. Fig 36). Respiratory changes, when present, were usually associated with stimulation within the reticular formation, in regions lateral to the obex. Sucking movements by the sheep were occasionally seen when the electrode tip was about 5 mm. deep and 1 to 3 mm. lat. to midline, about 6 mm. anterior to the obex, i.e. in the region of the nucleus ambiguus.

In contrast to the findings described above, particularly when stimulating in parts of the reticular formation 5 to 7 mm. anterior to the obex, stimulation would suppress a contraction of the reticulum which had already commenced although on cessation, an immediate contraction of the reticulum could be seen; sometimes the reticulum contracted several times in rapid succession (as in Fig. 39). These contractions lasted about the same time as the larger rhythmic contractions (about 10 secs), but the interval between them was only about 10 secs. and the amplitude was about half that of the regular contractions; they tended to be monophasic. Motility of this type was never seen following stimulation of the peripheral ends of the transected thoracic vagal trunks, but is very similar to that described by Dussardier (1960) following stimulation of the nucleus of the solitary tract; it appears to be reflex in origin. It differs

Fig. 39. 'Reflex' contractions of the reticulum following point electrical stimulation (1 V at 40 c.p.s. for 3 secs) of the medulla oblongata at the points shown on the bottom trace. The electrode tip was lying 3 mm. anterior to the obex, 2 mm. lateral to mid-line and 5 mm. deep - histological control showed that this was on the course of nerve fibres running from the dorsal vagal nucleus to the vagal rootlets. There is an immediate contraction of the reticulum and dorsal and ventral sacs of the rumen, followed by 2 or 3 additional contractions of the reticulum in rapid succession.


Reticulum
15 mmHg
5
Dorsal Sac
10 mmHg
5
Ventral Sac
10 mmHg
5
Time (mins.)
Stimulation

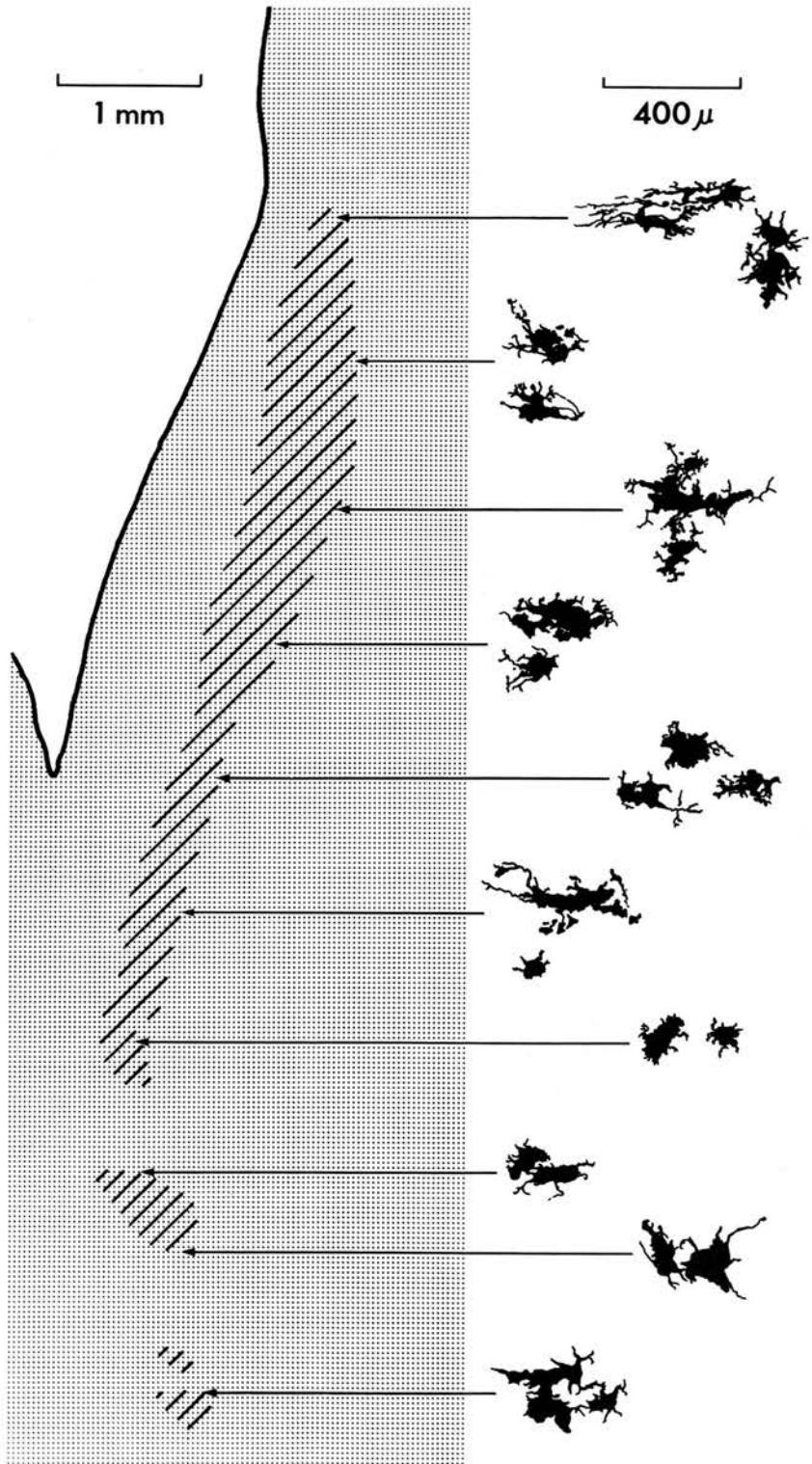
from the complex pattern of reticular contraction which sometimes occurs rhythmically in anaesthetised sheep, in that relaxation between each phase is complete, and each contraction is discrete.

If however, the stimulation was continued for up to 30 secs., the reticulum, after an initial interruption in its contraction, would contract in spite of continuing stimulation, and the amplitude of the pressure rise would be similar to that showed in the absence of stimulation. When the reticulum contracted immediately after cessation of stimulation, or during stimulation, subsequent reticular contractions usually showed a reduction in amplitude, and sometimes a drop in frequency. Stimulation on the course of fibres running from the dorsal motor nucleus to the vagal rootlets, caused contraction of both the reticulum and rumen. Even with reduction of the stimulus strength to 0.25 V, such responses were still easily and repeatably obtained. At intensities of 0.5 to 2 V, a series of smaller reticular contractions often followed cessation of stimulation, even if there had been no short latency reticular contraction. These resembled those described in the previous paragraph. In these preparations, where the reticulum and rumen were contracting rhythmically, these low amplitude contractions of the reticulum, which have been designated 'reflex' above, were seen more frequently than when the rumino-reticulum was not contracting.

Cyto-Architectonics of the Dorsal Nucleus of the Vagus

A summary of the histological observation is presented in Fig. 40. Preparations from the two different animals showed very similar characteristics, and the sizes of the cells at the various levels corresponded closely with those measured from slides subjected to the 'routine' staining with toluidin blue.

Fig. 40. The cyto-architectonics of the dorsal vagal nucleus as revealed by the Golgi-Cox impregnation technique. Outlines on the right show the shapes of neurons at different levels of the dorsal vagal nucleus (on the left) as seen on transverse section. Small cells (about 50μ) can be seen at all levels, and appear to form synapses with the larger cell bodies (about 100μ). There is an increased complexity of dendritic branching toward the anterior extremity of the nucleus.


1 mm
400 μ

There is no evidence that cells in the anterior part of the dorsal motor nucleus of the vagus are smaller than those lying posterior to the obex, as claimed by Szabo & Dussardier (1964), although this does not seem to be true for the hypoglossal nucleus which lies immediately below it. The complexity of dendritic branching, however, is considerably greater in the anterior part of the dorsal nucleus of the vagus, than it is more posteriorly, and the ramifications of the dendrites appear to extend over much greater distances. At the anterior extremity of the nucleus, many of the cells are elongated at right angles to the median raphe, so that they have a combed out appearance, lying along the plane in which axons can be seen emerging from the nucleus. This appearance is also described by Pattison & Holman (1943).

Small cells (about 50 μ) could be seen at all levels of the dorsal vagal nucleus and these appeared to form synapses on the larger cells (about 100 μ) at each level and also on other small cells. There is, therefore, no morphological evidence that the smaller cells are collected together, nor that they constitute a functionally distinct group, as might be inferred from Szabo & Dussardier's description.

Unit Activity in the Dorsal Nucleus of the Vagus.

It was very difficult to record unit activity from the dorsal nucleus of the vagus. Because of the relatively large size of the animal, respiratory and cardiovascular movement artefacts are correspondingly greater in amplitude than in the more usual laboratory animals such as rabbits or cats; attempts to record from the medulla oblongata, therefore, where movement tends to be most pronounced, present a considerable technical challenge. It is possible, for example, to prepare a pneumothorax, and to ventilate the animal artificially; when this was attempted, it was

found that motility of the reticulum rapidly declined.

Much of the movement associated with respiratory excursions could be eliminated by tilting the head sharply forward in the head frame, holding the medulla under a moderate degree of stretch. This procedure, however, did not alter the pulsation associated with beating of the heart. Attempts to minimise this excursion by placing a perspex foot on the surface of the medulla are frustrated by the anatomy of the region to be studied, which lies very superficially, and is placed at an angle on the floor of the fourth ventricle. The use of low-melting-point (39°C) paraffin wax, as advocated by Wall & Taub (1962) also proved ineffective. For reasons such as these, only 6 sheep from the total of 20 used in these experiments, provided satisfactory records, and the data presented were obtained from these 6.

Units were often lost after 1 to 2 contractions of the reticulum, and thus it was not possible to determine whether the discharge bore a constant temporal relation to each motility cycle. However, by plotting a frequency histogram for each burst of spikes recorded about the time that the reticulum contracted, it is possible to demonstrate that certain types of discharge show a consistent time relationship to the pressure rise recorded from the lumen of the reticulum. The results presented here apply only to activity patterns seen on four or more occasions. One hundred and four units are described and classified according to frequency of discharge, duration of activity, and the relationship of this activity to the contraction cycle of the reticulum (see Figs. 41, 42 and Table 1).

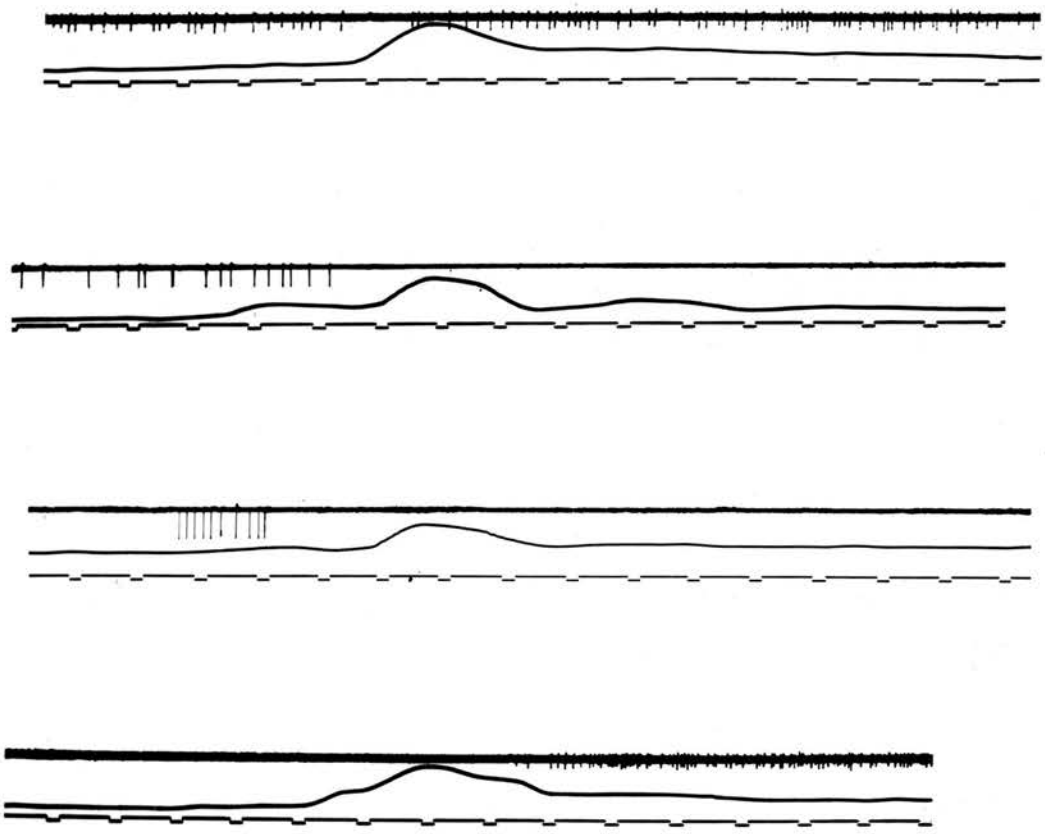
These units are classified by relating the time of appearance to the peak of the second reticular contraction. Stimulation of the peripheral end of the transected dorsal vagal trunk at a point 5 cm from the cardia causes the reticulum to contract after a latency of about 0.8 to 0.9 sec. (Fig. 6). Allowing a

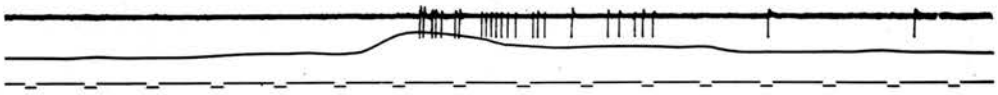
Figs. 41 and 42. Examples of unit discharges recorded from the dorsal nucleus of the vagus (some re-touched).

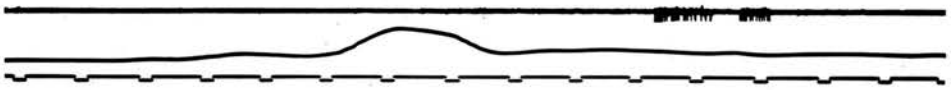
Upper - electrical activity picked up by the micro-electrode

Middle - pressure detected by a balloon in the lumen of the reticulum

Lower - 1 sec. time trace







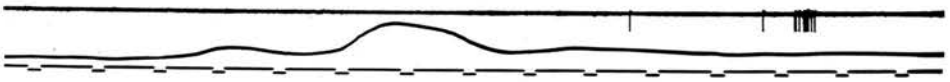
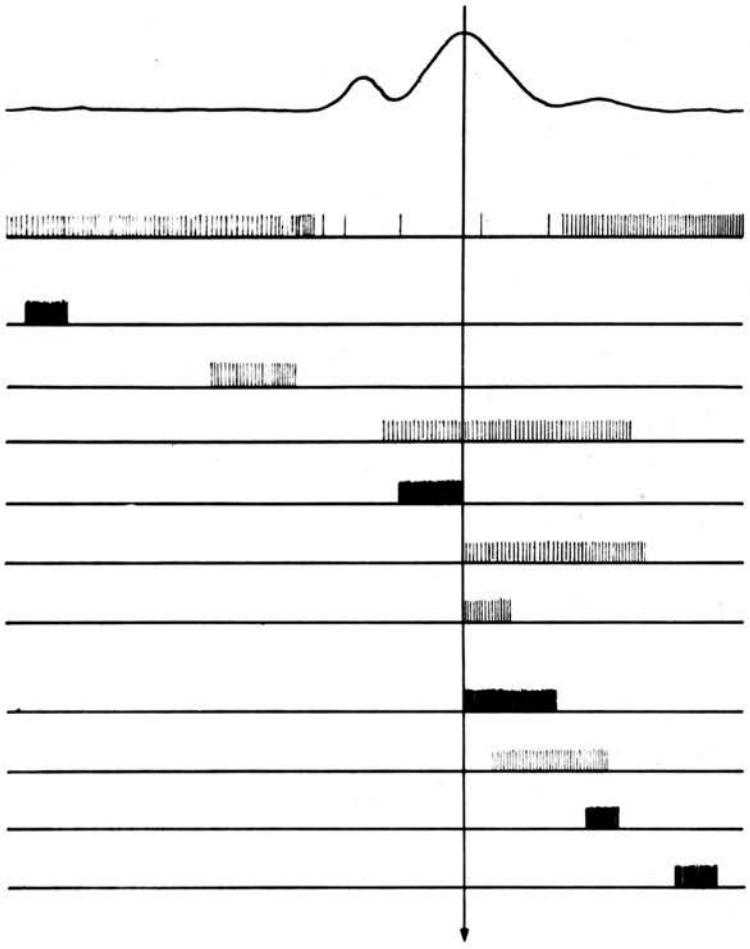


Table 1. Classification of the types of unit discharge recorded from the dorsal nucleus of the vagus, and showing modulation at the time of reticular contraction (upper left). Units are classified according to the duration and frequency of discharge and the time of onset relative to the peak of reticular contraction (vertical arrow). These patterns of activity are shown schematically on the left and are compared with patterns of discharge recorded from the cross-innervated diaphragm (Dussardier) or directly from vagal fibres (Leek).



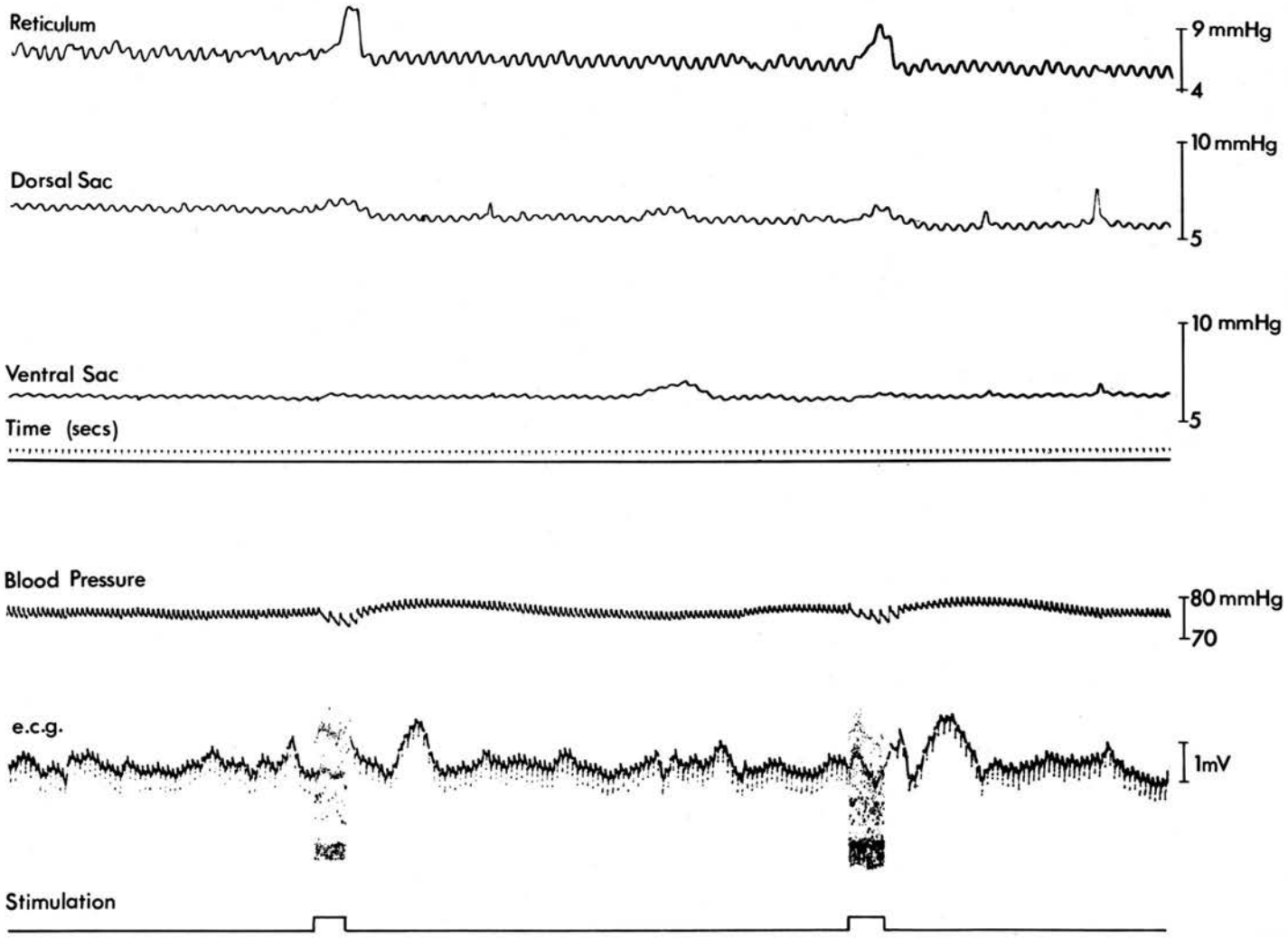
Unit	Dussardier type	Leek type	Onset of discharge relative to peak of 2nd reticular contraction	Duration of discharge (secs.)	Mean peak frequency of discharge	Number of units seen (104 observations
1	-	-	Spontaneous activity which ceases during contraction of reticulum		Up to 15/sec.	5
2	-	-	13-14 secs. before	1-2	60-90/sec.	5
3	-	-	6-10 secs. before	2-5	15/sec.	5
4	K	C	2.5 secs. before	4-13	Up to 15/sec.	26
5	C	-	1-4 secs. before	about 1.5	40-60/sec.	13
6	L	D	Simultaneous	3-12	10-20/sec.	12
7	D	-	From 1 sec. before to 2 secs. after	1-1.5	10-20/sec.	5
8	-	-	Up to 1 sec. after	1-4	Up to 50/sec.	6
9	F	-	1-4 secs. after	4-6	Up to 15/sec.	7
10	-	-	2.5-6 secs. after	1-2	60-100/sec.	8
11	-	-	5-10 secs. after	1-2	30-40/sec.	12

conduction time from the medulla to the cardia of 0.1 sec., the interval between excitation of the vagal rootlets and the onset of contraction of the reticulum is about 1 sec. A further correction of 1 sec. is required to allow the contraction to reach its peak (see Fig. 43). The total correction factor, to allow for conduction and contraction times, is therefore approximately 2 sec. This calculated value agrees well with measurements of the latency of the peak of the reticular contraction with stimulation of reticular motor fibres within the medulla (Fig. 43). A discharge which is recorded while the second reticular contraction is at its peak (e.g. type 6 in Table 1) would not arrive at the stomach until 2 sec. afterwards.

Very few units showed a resting discharge - the 5 units which did appear to be firing 'spontaneously' (type 1) showed a drop in frequency of discharge, or absolute quiescence, during the first and second phases of contraction of the reticulum - resting discharge of these units was 15/sec. or less and the spike frequency remained depressed for periods of 3 to 7 sec. during the period while the reticulum was contracting.

The commonest type of activity recorded (26 units - type 4) was a prolonged discharge lasting 4 to 13 sec. at a frequency of about 10 to 15 per sec. beginning early during the first phase of contraction of the reticulum, (within 1.5 sec.); discharge frequency of such units was often irregular; frequently bursts (particularly shorter ones, lasting up to 7 sec.) were biphasic - in such cases the two peak frequencies usually occurred about 2 sec. before the peaks of the respective reticular contractions. Another pattern of activity frequently seen during the first contraction of the reticulum was a high frequency (40 to 60 per sec.) burst of spikes lasting approximately 1.5 sec. - discharge patterns of this type were recorded on 13 occasions (type 5).

Fig. 43. The response to point electrical stimulation of the medulla oblongata on the course of vagal fibres leaving the dorsal vagal nucleus. 10 msec. pulses of 0.25 V intensity at 50/sec were delivered from a Grass SD5 fully isolated stimulator to a glass - Wood's metal electrode implanted stereotaxically. On this occasion no reflex contractions appeared. The latency of the reticular contraction is about 2 secs.


Reticulum
9 mmHg
4
Dorsal Sac
10 mmHg
5
Ventral Sac
10 mmHg
5
Time (secs)
Blood Pressure
80 mmHg
70
e.c.g.
1mV
Stimulation

A further type of unit (12 cases - type 6) often began to fire at the peak of the second contraction of the reticulum at a frequency of 10 to 20 per sec., remaining active for 3 to 12 sec., whereas higher frequency bursts of about 50 per sec., lasting 1 to 4 sec., were sometimes (6 occasions) seen up to 1 sec. after the peak of this contraction (8, Table 1). Additional discharge patterns seen about this time were: a low frequency (10 to 20 per sec.) discharge lasting 1 to 1.5 sec., and appearing up to 3 sec. after the beginning of the second reticular contraction (type 7 - 5 units), and a more prolonged discharge lasting 4 to 6 sec. at 15/sec., beginning 1 to 4 sec. after the peak of the contraction (type 9 - 7 units). High frequency activity (60 to 100 per sec.) was also sometimes seen (8 times), commencing 2.5 to 6 sec. after the peak of the second reticular contraction, and lasting 1 to 2 sec. (type 10). Short (1 to 2 sec.) discharges at a slightly lower frequency (30 to 40 per sec.), and beginning 5 to 10 sec. after the peak of this contraction, were recorded on 12 occasions (11, Table 1).

Activity sometimes appeared before the reticulum began to contract, although such cases were in a minority - two types were seen on 5 occasions each: a high frequency burst (60 to 90 per sec.) beginning 9 to 10 sec. before the reticulum began to contract, and lasting 1 to 2 sec. (type 2) and a much slower discharge at about 15/sec., commencing 2 to 6 sec. before the pressure rise first became apparent in the reticulum, and lasting 2 to 5 sec. (type 3).

A total of 14 units which became active about the same time as the reticulum contracted, has been discarded in this description, because their pattern of activity was not repeated sufficiently to establish the criteria laid down earlier, although it is possible that some of these may have been functionally associated with contraction of the forestomach - 3 of these

units, for example, discharged at a frequency of 50 to 60 per sec. for 1 to 2 sec. up to 2 sec. before the pressure within the reticulum began to rise.

Unit activity of the type presented here was only recorded from that part of the dorsal vagal nucleus extending between the level of the obex and 4 mm. anterior to this. Although the entire length of the dorsal nucleus posterior to the obex was examined systematically, unit activity showing consistent modulation at the time of a forestomach contraction was never observed here. In two experiments, systematic attempts were made to record neural activity related temporarily to a gastric contractile event, from the reticular formation of the medulla oblongata; in neither case were such units detected.

Although most units were 'silent' by the time the pressure within the reticulum had returned to the 'resting' level, a few (see e.g. 6, 9, 10 & 11, Table 1) continued to discharge.

In some cases this unit activity could be correlated temporally with contraction of the dorsal sac of the rumen (the ventral sac had usually finished contracting before the dorsal sac), but occasionally the discharge continued after the rumenal contraction had ceased - if an additional conduction time of 2 sec. is allowed, it is quite clear that these discharges must be associated with activity of some other part of the stomach.

Often, contractions of the dorsal sac of the rumen were complex, resulting in two or three fluctuations of the pressure recorded from its lumen. No corresponding modulation of this 'late' unit activity occurred at this time, although it will be apparent from section 1 that it is important to be very cautious in analysing complex pressure wave-forms from the stomach, as different parts of the organ may be involved on each occasion.

DISCUSSION

Neurons showing evidence of chromatolysis following section of either the dorsal or ventral trunk of the vagus are confined to that part of the dorsal vagal nucleus lying anterior to the obex, and to the posterior part of the inferior ganglia of the vagus. These findings conflict with those presented by Szabo & Dussardier (1964), who failed to find any evidence for a somato-topographical distribution of degenerating nerve cells at either site, following section of the same nerves. The reason for this discrepancy in results remains obscure, and is unlikely to be explained by the fact that the French workers stained their sections with thionin, as this stain has been widely used for the demonstration of Nissl material. It has, however, been pointed out by Molhant (1911) that the chromatolysis which follows section of the respective axons is often less distinct and much more variable than that seen when the lesion is made by tearing the nerve across, as was done in the present study, and if this is in fact the case, it might be expected that the interpretation of results following nerve section would be considerably more hazardous. Photographs presented by these workers show very distinct degenerative changes, although they do report that cell changes were not generally very marked. Other factors may also be involved.

The results presented, however, agree well with those of Bell (1960) and Kitchell at al (1956), although these workers have only examined the alterations induced in the brain stem itself and have not investigated the distribution of degenerating cells of the superior or inferior ganglia of the vagus. Since the criteria adopted for chromatolysis are medium to severe on the scale proposed by Campbell & Novick (1946), it appears that sufficient degeneration had occurred to make accurate counts

feasible. It seems reasonable to conclude that the bulk of evidence available at present indicates that the dorsal vagal nucleus represents a final common pathway for motor fibres running to the ruminant forestomach, and that the neurons concerned with innervating this organ are confined to that portion anterior to the obex.

Low intensity point electrical stimulation of this region of the medulla oblongata, however, failed to elicit contraction of the rumen or reticulum. This indicates that either the stimulation parameters used were inadequate to excite sufficient numbers of motor cells to cause a detectable contraction, or alternatively that electrical stimulation of the dorsal vagal nucleus established an inhibitory state, which could prevent the contraction from occurring. This latter hypothesis appeared particularly interesting when considered in relation to Szabo and Dussardier's observation (1964) that cells in this region can be morphologically divided into two distinct types. The findings of these workers, however, did not accord with those of earlier workers (See e.g. Vermeulen, 1913-15; Pattison and Holman, 1943 etc.), and the Golgi-Cox histological preparations discussed earlier failed to confirm the existence of two morphologically distinct types of cells within the anterior part of the nucleus.

It is possible to stimulate efferent fibres leaving the dorsal vagal nucleus; responses obtained from the paths of such fibres had a short latency, occurred almost simultaneously in reticulum and dorsal, and ventral sacs of the rumen, and if stimulation was continued, reticular pressure began to fall within a few seconds; all these features are also seen on stimulation of the peripheral stump of the transected cervical vagus. In addition, stimulation at these positions was often followed by a delayed series of contractions of the reticulum, beginning after a greater latency and continuing after cessation of stimulation - these responses resemble those seen following stimulation of the central end of the ventral thoracic vagus, or of the abdominal nerve, and are

probably reflex in nature, similar to those observed by Dussardier following stimulation of the nucleus of the Solitary tract.

Only on 2 occasions were excitatory responses observed from regions other than those directly on the course of vagal fibres running through the medulla, and in both these cases the electrode was situated a considerable distance away from the dorsal nucleus of the vagus. It is possible that these latter responses resulted from activation of fibres or cells from remote parts of the body, as Dussardier (1955) has commented on the possibility of eliciting gastric contractions by stimulation of the sciatic nerve in sheep, and Borgatti and Matscher (1963) have investigated in some detail the inhibitory and excitatory effects on the reticulo-ruminal centre, of stimulation of the mandibular nerves.

Although focal electrical stimulation (at 0.25 V) of vagal efferent fibres within the medulla could evoke clear rises in pressure within the rumen and reticulum, stimulation of the nucleus at 2 V, with all other parameters unchanged, failed to cause any alteration in pressure within these compartments, so that it is unlikely that the absence of a detectable contractile response in the latter instance is due to inadequate stimulation. The fact that stimuli of up to 15 V did not cause any response, even though delivered along the whole extent of the nucleus, anterior to the obex, provides further evidence that failure to record a contraction was not a result of failure to excite sufficient numbers of motor nerve cells.

Evidence for an inhibitory mechanism within the dorsal nucleus of the vagus was obtained, however, by focal stimulation during rhythmic contractions of the rumino-reticulum. The predominant effect elicited during these experiments was an inhibition of rhythmic contractions, with reduction in both amplitude and frequency of the contractions of the rumen and reticulum. This inhibition can also be demonstrated following

focal stimulation at points outside the dorsal nucleus of the vagus, i.e. from regions extending anteriorly and ventrally from the mid-region of the nucleus. This can be seen quite clearly when a 'contour map' is constructed, joining points from which similar inhibitory responses can be elicited without alteration of stimulation parameters. Contours of this type, whether plotted to demonstrate the effect of stimulation on amplitude or frequency of the contractions of the reticulum, show a very similar disposition; there is, at this stage, no need to postulate the existence of separate elements to explain the changes in frequency and amplitude of contraction. Histologically, there are no special accumulations of cell bodies along the axis of these contours, and it is therefore possible that stimulation was activating inhibitory fibres passing down to the dorsal nucleus of the vagus from higher levels of the encephalon.

If it is assumed that the inhibitory action is exerted within the dorsal nucleus of the vagus, as shown in Fig. 38, the results obtained by destroying different parts of the nucleus (Beghelli et al, 1964) become rather interesting. For example, bilateral destruction of the anterior extremity of the nucleus abolished all rhythmic motility of the reticulum, whereas destruction of those parts of both nuclei lying just anterior to the obex did not necessarily prevent rhythmic motility. Unilateral lesions involving either nucleus, anterior to the obex, failed to abolish contractions. The experimental evidence presented here suggests that there are powerful inhibitory mechanisms in that region of the vagal nucleus between the obex and 3 mm. anterior to it, i.e. that part of the nucleus which cannot maintain rhythmic gastric motility in the absence of the most anterior part of the nucleus.

The recordings of unit neural activity from the dorsal nucleus of the vagus provide conclusive evidence that this part of the brain stem is involved in the periodic initiation of

contractile cycles of the reticulo-rumen, and, moreover, that only that part of the nucleus lying anterior to the obex is involved in this function. Data obtained during the studies involving chromatolysis of the neurons in the area, indicated that the sum of the degenerative changes induced in this part of the dorsal nucleus following section of dorsal and ventral vagal trunks is insufficient to account for all the cells occupying the anterior part of the nucleus. These cells could be involved in innervating other parts of the body, or they could be neurons resistent to chromatolysis changes, or alternatively they could represent inter-neurons. Unfortunately, there is no information available to help distinguish between these three possibilities, although Bell reports complete cellular degeneration following a high cervical vagotomy, whereas Dussardier found that some cells did not degenerate after high vagal transection. One must, therefore, avoid the assumption that all unit activity recorded from this part of the vagal nucleus necessarily represents traffic destined to pass down the vagus to the stomach. There is, in this context, the added complication that it would theoretically be possible to record electrical activity from nerve terminals rather than from neurons, although the duration of the 'spikes' (1 to 2 msec.) and their shape, suggests that the event being recorded has the electrical characteristics of an action potential rather than an asynchronous volley of such impulses in the branches of nerve endings.

A comparison of the unit population with that presented by Dussardier (1960) shows that many of the units seem to be of the types described by him; there is, however, a slight difference in frequency values quoted, although this is probably due to the fact that units described in this thesis were analysed over intervals of 0.15 sec., i.e. almost instantaneously, whereas Dussardier appears to have analysed over somewhat longer intervals, and hence his values appear to be a little lower.

When this is taken into consideration, it will be noticed that five of the eleven patterns of activity listed can be fitted into discriptions by Dussardier, of efferent traffic down the thoracic vagus as translated by re-innervated diaphragm musculature.

B.F. Leek (personal communication) has also accumulated data on the types of efferent unit discharges which can be recorded from the cervical vagus of anaesthetised sheep at the time of reticular contractions. The system of classification adopted by this worker differs from the one adopted here, in that distinction is made primarily on the basis of the pattern of discharge rather than frequency of time relationships of activity. Leek does, however, describe biphasic bursts of activity at frequencies of up to 15/sec which correspond in time of onset and duration to type 4 presented here, and he also describes units which appear to correspond to type 6.

Of the units described in the present work, types 4, 5, 6, 7 and 9 closely resemble activity described by Dussardier and Leek, and these may be presumed to represent efferent outflow; this accounts for 66% of all units presented here, and accounts for 90% of all phasic units with a peak discharge frequency of less than 30/sec. These observations suggest a further classification into frequency of discharge, low frequency units being efferent in nature, higher frequency units apparently not contributing to activity within the vagal trunks, and hence presumably being involved in central processes - i.e. as interneurons. An alternative possibility is that high frequency discharges are not accurately translated by Dussardier's preparation; further evaluation requires great caution.

One very interesting type of discharge pattern is that tabulated as 1, in Table 1, in which a neuron showing a continuous spike discharge at low frequencies becomes quiescent during contraction of the reticulum. That this is not

a sensory cell is suggested by its location, within a nucleus which is ontogenetically motor in origin, and for which there is no evidence of a sensory component, and also by the fact that on two occasions the discharge rate began to fall before any rise in pressure was observed in the reticulum. As there is no evidence of the existence of in-parallel receptors, in the fore-stomach wall, it would be very difficult to explain this behaviour on the basis of a sensory input to the nucleus. It is tempting to speculate on the nature of these 5 units, in relation to the pronounced inhibitory effects revealed by focal electrical stimulation, as it would be possible to predict the existence of such neural activity. If this pattern of activity does represent a restraining influence on the centre, 'release' of which coincides with the initiation of a gastric contraction by the reticulo-ruminal centre, a rough parallel with Delisle-Burns' and Salmoiraghi's concept of the respiratory centre is possible. Both respiration and forestomach motility are periodic events, capable of being markedly influenced by other parts of the central nervous system, and also by various different influences from the 'periphery'. In each case, the response to sensory input may resemble that predicted on the basis of cybernetic 'feed-back' systems, although the two systems appear to show a close functional liaison with the reticular system of the brain stem. Functional centres controlling rumino-reticular motility and respiration are both present in that part of the medulla lying between the level of the obex and the posterior border of the pons, although the activity of each is reduced as successive transverse sections from the posterior mid-brain are made at progressively more posterior levels.

Unfortunately, the neuronal analogue of an electrical flip-flop circuit, advanced by Salmoiraghi to explain the properties

of the respiratory centre, in its basic form, will not explain the great disproportionality between the two states of the reticulo-ruminal motor centre. Under normal conditions, the relatively close relationship between contraction of the reticulum and of the rumen suggests (assuming that each stage of the motility cycle is controlled by the centre), that the centre responsible for the contraction of the reticulum is directly responsible, in turn, for activating the corresponding 'ruminal centres'. If this is so, or if subsequent parts of the contraction cycle are initiated peripherally, the neuronal activity responsible for the contractile process could not operate as a state of a flip-flop arrangement. The time factors in any such circuit would thus consist of about 6 secs. in the 'on' state, followed by 54 secs. off (i.e. the time spent by the reticulum in the "contracted state" relative to that in the relaxed state). The unit activity recorded from the dorsal nucleus of the vagus does not occur in synchronised bursts - in fact 11 different types of unit discharge have been recorded.

No unit activity showing modulation at the time of gastric contraction has been recorded from other parts of the medulla. Evidence presented in this thesis suggests that the principal, if not the only centre controlling gastric motility in the ruminant, occupies the region of the anterior part of the dorsal vagal nucleus. Within this area, there is no electrophysiological evidence for synchronised bursts of activity such as would be expected if excitation and inhibition alternated with one another - a basic requirement of the flip-flop hypothesis. However, while the mode of operation of the centre remains obscure, it seems probable that a more extensive analysis of unit activity in the dorsal nucleus of the vagus would make a very profitable investigation.

CONCLUSIONS

Findings described in this thesis provide clear evidence that the reticulo-ruminal motor centre lies posterior to the level of the colliculi, in the brain stem. The output from this centre is passed via the vagi to the rumino-reticulum, which undergoes a cycle of contraction. Data obtained from the experiments described do not decisively indicate the extent to which the centre is concerned with coordinating contractile events in each motility cycle, but much of the evidence collected suggests that there is some coordination at the gastric level.

Preganglionic vagal efferent fibres, supplying the ruminant stomach, are derived from that part of the dorsal vagal nucleus situated anterior to the obex. Cells here have longer and more complexly branched dendrites than those at other levels of the nucleus. The cell bodies of gastric afferent vagal fibres lie in the distal part of the inferior vagal ganglion. Powerful inhibitory mechanisms exist in the region of the dorsal vagal nucleus, and these can completely inhibit gastric motility; more anterior levels of the brain stem are also capable of depressing rumino-reticular motility, probably also by an effect on this part of the dorsal vagal nucleus.

Unit neuronal activity can be recorded from the anterior half of the dorsal vagal nucleus using metal-filled glass micro-electrodes. Many of these neurons become active about the time that the reticulum contracts. Only about 5% of the units studied showed a standing discharge - in these cases the spike frequency fell during reticular contraction; such activity could be involved in the inhibitory mechanism. Discharge patterns of several of the units recorded are quite unlike activity reported to exist in the vagus nerve, and it is likely from the characteristics of these that they represent intermediate stages in the genesis of traffic destined for the vagus.

APPENDIX

The experiments described in Section 2, performed to determine the effects of section of the vagal trunks on motility of the rumino-reticulum, involved the calculation of a large series of mean values and standard deviations from batches of data obtained at different times. The mathematics required by this analysis was performed on an Atlas computor, from a program written in Atlas autocode. Sets of data are punched onto tape, and each block of data is terminated by the symbol -1. When all the figures have been thus prepared, the complete program is terminated by the symbol -2, followed by the usual ***Z. Print-out is provided in 3 columns headed mean, S.D. and n (i.e. number of data/block).

The program used was as follows:

```
begin
real array A (1:80)
integer n,i
real x, a, j
3: n=0
a=0
j=0
4: read (x)
-> 2 if x=-1
-> 1 if x=-2
n=n+1
A(n)=x
a=a+x
-> 4
2: a=a/n
cycle i=1,1,n
A(i) = (A(i)-a)^2
j=j+A(i)
repeat
newline
caption mean£=£
print (a,3,2)
Caption£££££n£=£
print (n,3,0)
caption£££££S.D.£=£
print (sqrt(j/(n-1),3,2)
-> 3
1: end of program
```

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